VOLUME 47 JUNE, 1957 NUMBER 6

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

UNIVERSITY OF MICHIGAN JL 22 1957 SCIENCE LIBRARY

CONTENTS

Cornelius Lott Shear — John A. Stevenson 321

Grain storage studies. XXIV. Moisture content of wheat seed in relation to invasion of the seed by species of the Aspergillus glaucus group, and effect of invasion upon germination of the seed — John F. Tuite and Clyde M. Christensen 323

Analysis of Fusarium resistance in tomato by grafting experiments — R. P. Scheffer 328

The effects of some leaf blights of corn on grain yield — Arnold J. Ullstrup and S. R. Miles 331

Osmotic pressure and plant virus local lesions — James D. Panzer 337

Fusaric acid as a wilt toxin — Ernst Gäumann 342

Relation of fungicide and miticide treatments to winter injury and spring blackspot development on roses — W. D. McClellan, Edgar A. Taylor, and Floyd F. Smith 357

Effects of X rays on the germination of conidia of the oak wilt fungus — Bert M. Zuckerman 361

Effects of genetic segregation in Phaseolus vulgaris on the symptoms induced by bean yellow mosaic virus — James R. Baggett 365

The isolation of the toxin of Helminthosporium victoriae — Ross B. Pringle and Armin C. Braun 369

Biophysical studies of wild cucumber mosaic virus — J. B. Sinclair, P. H. Gell, and Paul Kaesberg 372

A Phytopathological Note:

Organic acids and the control of two seedling blights by the water-soak method — P. Jacquet, Curt Leben, and D. C. Arny 377

News

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CORNELIUS LOTT SHEAR, 1865-1956

John A. Stevenson

Cornelius Lott Shear, botanist, mycologist, plant pathologist, died in Monroe, Louisiana, on February 2, 1956, after a long illness. Dr. Shear was born March 26, 1865, at Coeyman's Hollow, near Albany, New York, the son of Henry and Mary (Speenburg) Shear.

He graduated from the New York State Normal School at Albany in 1888 and taught in secondary schools for some years before entering the University of Nebraska in 1894. From this institution he received the B.S. degree in 1897 and the M.A. in 1901. The Ph.D. degree was conferred by George Washington University in 1906, Dr. Shear's studies of cranberry diseases forming the subject matter of his dissertation.

During his undergraduate years he served in the summer months as a special field agent of the Division of Agrostology of the United States Department of Agriculture and from 1898 to 1901 as an assistant agrostologist with the same Division. A number of Department publications recorded his taxonomic work with the genus *Bromus* and his field studies of western grasses and other forage plants. Since his early youth in New York, where he had been encouraged in the study of fungi by Charles Peck, the State Botanist, and E. A. Burt, one of his Normal School instructors, his real interest had been with this group of plants, and with the establishment of the Bureau of Plant Industry in 1901 he seized the opportunity to join the famous Galloway team of "vegetable pathologists."

Cotton root rot, a disease then rampant in Texas, was his first assignment, and he gave particular attention to its etiology and to a search for control measures. He named the causal organism for the first time and in collaboration published several papers on the nature of the disease and the results of field experiments.

Shear was soon transferred to the small fruit disease project and shortly thereafter became its head, a position retained until his retirement. To his duties in this position there was added in 1923 headship of the Division of Mycology and Disease Survey, formed by the consolidation of the offices of the Plant Disease Survey and Pathological Collections of the Bureau of Plant Industry.

In the cranberry disease work, he early established a reputation as a careful and effective worker and was a welcome figure at national and local meetings of cranberry growers. Either alone or in collaboration with N. E. Stevens and others, he worked out the etiology of the various fungus diseases of this crop and studied intensively the relation of cultural methods, fungicidal sprays, and other factors to disease control. More than 25 papers covered his contributions in this field.

Nor were other small fruit diseases neglected. He worked intensively on black rot and other grape diseases, always giving particular attention to the life histories of the fungi concerned. General treatises on diseases of grape, cranberry, and other small fruits were published in the Farmers' Bulletin series of the Department.

CORNELIUS LOTT SHEAR
1865–1956

With the coming of the chestnut blight scourge, Shear was detailed to study the causal fungus, to work out its life history, and to determine whether it was in fact an introduced species or an American species "gone wrong." Here again in collaboration with Stevens and others, he definitely settled the mycological problems involved. The bulletin on *Endothia parasitica* and related species is a classic in mycological research.

As a result of the work of himself and associates with strawberries and cranberries in particular, he early came to appreciate the enormous losses that occurred between the time of picking the crop and its use by the consumer. Several papers were published calling attention to these facts and the need for action. Preliminary steps taken by federal and state governments to meet the situation were discussed and the great possibilities of saving food during wartime scarcities emphasized.

Shear's extra-curricular activities were many. He

PHYTOPATHOLOGY for May (47:247-320) was issued June 17, 1957

was interested in Botanical Abstracts and its successor, Biological Abstracts. He was long concerned with stability and uniformity in botanical nomenclature and took an active part in several international botanical congresses. His accomplishments in this field are recounted elsewhere (Taxon).

He was not only a charter member of the American Phytopathological Society but the actual individual who took the necessary steps to crystalize the feeling of many phytopathologists of the time and to get the Society under way. In his history of the first decade of the Society (Phytopathology 9: 165-170. 1919.) he relates the story of its founding in cooperation with W. A. Orton, Donald Reddick, L. R. Jones, and others. He served the Society as Secretary-Treasurer for 9 years, as a member of the Board of Editors, and as President in 1919. He did much to shape the policies of the infant organization and maintained a deep and abiding interest in the progress of the Society and matters phytopathological throughout his life.

His voluminous correspondence with other phytopathologists of his time, which covered a wide range of topics from the general needs of the science to specific discussion of one or another plant disease, will constitute most useful source material to the future historian who would write on phytopathology.

He helped establish the Journal of Agricultural Research in 1913 and was an active participant in its affairs as a member of the editorial board during the years 1925–1930. During this time it was the favorite medium for publication of much of the phytopathological research of this country, including a considerable amount of his own work such as Shear and Dodge on "Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group," the paper that launched this ubiquitous fungus into genetic fame.

The need for a bringing together of pure cultures of fungi and other organisms for use in pathological research, mycological comparisons, and other obvious purposes became evident to him early in his career, and he finally had a hand in the setting up of the American Type Culture Collection. He served on the Board of Directors of this organization for many years.

Shear was an active member of many other scientific societies. The Botanical Society of America extended him membership soon after he came to Washington in a day when membership in the Society was limited to the "elect." He was its Vice-President in 1908. He was a charter member of the American Mycological Society and its Secretary-Treasurer until it combined with the Botanical Society of America in 1906. In later years he was a charter member and second President of the Mycological Society of America. Among other affiliations were the Botanical Society of Washington (President 1914), American Society of Naturalists, History of Science Society, Sigma Xi (President, Washington Chapter), American Association for the Advancement of Science (Fellow), British Mycological Society, and Washington Academy of Sciences. He was an official delegate to the second International Botanical Congress, Vienna, 1905, to the fourth, Ithaca, 1926, and to the fifth, Cambridge, 1930. He represented the United States at the International Phytopathological Congress in Holland 1923, and at the Pasteur Centennial Celebration at Strassbourg and Paris in the same year.

Dr. Shear married Avis Sherwood at Osborne, Kansas, in 1890, and for nearly 60 years thereafter she was the perfect helpmate, his constant inspiration, and a kind but firm codirector of Shearian activities. She passed away in 1950. The children, all of whom survived their parents, are Sherwood W. of Berkeley, California; Deming J. of Falls Church, Virginia; G. Myron of Blacksburg, Virginia; Cornelius B. of Gainesville, Florida; Mrs. Beatrice Cretney of Monroe, Louisiana; and Mrs. Dorothy Miles of Vienna, Virginia. There are 14 grandchildren and 3 great-grandchildren.

Dr. Shear was timid and retiring in his boyhood and early youth, as he himself has related; at all times, he was quiet, modest, and unassuming. His interests in life were his family and his scientific activities. He preferred the scientific activities to administrative responsibilities which came to him after he became a Division Head in 1923, and he soon developed a technique to avoid such interruptions and related extraneous matters by ignoring them or turning them over to a staff member to handle as he saw fit. He lived in a world of books, separates, papers, culture tubes and other glassware, a microscope, the original hand lens acquired as a small boy, and above all, specimens—specimens everywhere, loose on tables, heaped up on chairs, tucked into a miscellaneous collection of pasteboard boxes, and piled on top of every available piece of furniture to the uttermost reaches of a distant ceiling. All attempts to bring order to this mass of scientific material by others, or even to explore it, were definitely frowned upon, but the good Doctor himself could locate with a long arm any letter, specimen, or other item needed at a moment's notice.

Following retirement in 1935, he kept up his interest in plant diseases and the fungi, collecting in Florida during the winter months and at other times studying his material and writing up accumulated data at his office in Washington, until World War II forced the removal of the mycological collections to Beltsville.

Dr. Shear's publications over a period of nearly sixty years, his services to technical societies, and his wise counsel to growers, associates, and professional colleagues give him an assured place in the history of plant pathology and mycology.

GRAIN STORAGE STUDIES. XXIV. MOISTURE CONTENT OF WHEAT SEED IN RELATION TO INVASION OF THE SEED BY SPECIES OF THE ASPERGILLUS GLAUCUS GROUP, AND EFFECT OF INVASION UPON GERMINATION OF THE SEED [1]

John F. Tuite [2] and Clyde M. Christensen [3]

SUMMARY

Seeds of various samples of wheats nearly or entirely free of fungi were inoculated with 4 species of the *Aspergillus glaucus* group known to be prevalent on commercially stored grain, and stored at moisture contents of 12.2–16.0 per cent and 25°C for 1–15 months. At a moisture content of 13.0–13.6 per cent, seeds were invaded gradually by *Aspergillus restrictus* and *A. amstelodami* but slowly by *A. repens* and *A. ruber*. At moisture contents of 14.3–14.6 per cent, *A. repens* invaded a larger percentage of seeds in 2 months than did the other species, but within 4 months all fungi had invaded 100 per cent of the seeds. At moisture contents of approximately 15.5 to 16.0 per cent, 100 per cent of the seeds were invaded within 1 month. Germination percentage of the seeds inoculated with the various storage fungi decreased much more rapidly than did germination percentage of the noninoculated controls. *A. restrictus* appeared to be especially pathogenic, and its invasion was limited almost entirely to the germs of the seeds, although the other 3 species also attacked the germs preferentially. The upper limit of moisture content for long time storage of wheat, at temperatures that permit invasion by storage fungi, would appear to be approximately 13.0 per cent.

Invasion of stored wheat by species of *Aspergillus* is known to result in decrease in germination percentage and in the development of brown to black germs, a condition known in the trade as germ-damaged or sick wheat (3, 9). Rather extensive isolations over a period of several years from some hundreds of commercial parcels of stored wheat, in some of which deterioration had occurred, indicated that *Aspergillus amstelodami* (Mang.) Thom & Church, *Aspergillus repens* (Cda.) D By., *Aspergillus ruber* (Spieck & Brem.) Thom & Church, and *Aspergillus restrictus* G. Sm. were among the most prevalent storage fungi in such grain. It seemed desirable, therefore, to investigate more fully the lower limits of moisture content that would permit invasion of wheat seed by these fungi and the effect of such invasion on germination of the seed. Germination itself is not known to be a quality factor in wheat to be used for milling, but it appears to be a sensitive indicator of beginning or impending deterioration (9), and decrease in germination percentage always precedes the development of sick wheat (3). It is important to know the lower moisture content limits that permit invasion of stored seed by fungi because, in practice, much wheat is stored at moisture contents that permit invasion and deterioration by these fungi. The present federal regulations (11) specify an upper moisture content limit of 14.5 per cent for hard red spring and durum wheats and 14.0 per cent for all other classes of wheat. There is some evidence that these limits are too high for long-time storage (2, 7), but more extensive and detailed data on this point are needed.

MATERIALS AND METHODS.—Wheats of different classes and from different sources were used. All were from lots which preliminary tests had shown to be almost free of Aspergillus or other fungi capable of growing in seed at moisture contents below 18 per cent. In all tests the seeds were shaken for 2 minutes in a 1 per cent solution of sodium hypochlorite. A more concentrated solution of sodium hypochlorite, or a longer period of treatment, reduced germination percentage of the seed after storage for some months. After this treatment, the seeds were rinsed with sterile water and dried aseptically. The moisture content of each lot was determined after drying, and sterile water of the amount needed to increase the moisture content to the desired level was added. The seeds then were stored in a sterile container in a refrigerator for 5 days and shaken at intervals to insure even distribution of water. From 2 to 6 replicates of approximately 10 g each were prepared for each individual storage period and treatment. This amount was used because it was found difficult to keep larger quantities of seed free of storage fungi for as long as several months.

Fungi used to inoculate the seeds were isolated originally from wheat taken from commercial bins. Inoculum was prepared by autoclaving wheat at approximately 18 per cent moisture and inoculating small lots with each individual fungus. Five such seeds, with the fungus fruiting abundantly on them, were used for each individual inoculation. The inoculated seeds were stored in cheesecloth bags above

[1] Accepted for publication January 10, 1957.
Paper No. 3691, Scientific Journal Series, Minnesota Agricultural Experiment Station.
The work here reported formed a portion of a thesis presented by the senior author to the Graduate School, University of Minnesota, in partial fulfillment of the requirements for a Ph.D. degree. The work was supported in part by grants from Cargill, Inc., Minneapolis, Minnesota. The writers are indebted to various members of Cargill, Inc. for samples of grain and for other aid.

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[3] Professor, Department of Plant Pathology and Botany, University of Minnesota.

saturated salt solutions in 8-oz. French-square jars. The salt solutions, the corresponding relative humidities (r. h.) they presumably maintained at 25°C, and the authorities for each were as follows: r.h. 65 per cent, sodium nitrite (12); r.h. 71 per cent, a combination of ammonium chloride and potassium nitrate (6); r.h. 76 per cent, sodium chloride (12); and r.h. 80 per cent, ammonium sulphate (12). That these solutions actually did maintain the relative humidities they were supposed to is indicated by the fact that in all cases the moisture contents of the samples when tested during storage agreed, within the range of experimental error (about plus or minus 0.3 per cent) with the moisture contents supposed to be in equilibrium with each (5). Moisture contents were determined on samples at the beginning of each test and again at the end of the first storage period, 1–4 months later. Moisture contents were determined by the 2-stage, air-oven method specified by Cereal Laboratory Methods (1) and are given on a wet-weight basis.

The number of seeds infected by various fungi was determined by immersing and shaking 100 seeds for 1 minute in 1 per cent sodium hypochlorite, rinsing them in sterile water, and culturing them on malt agar containing 7.5, 15, or 20 per cent sodium chloride. Preliminary tests had shown that when *A. restrictus*[4] was present in seeds, along with other members of the *A. glaucus* group, it frequently could be detected only if seeds were cultured on a medium containing 15–20 per cent sodium chloride. On acid potato-dextrose agar, it grew very slowly or not at all; on malt agar containing 7.5 per cent salt, other species of the *A. glaucus* group often overgrew and concealed it. Germination percentage of the seed was measured by placing 100 seeds on moist paper at room temperature and counting the germinated seeds after 6 days; usually several replicates of 100 seeds each were so tested at each storage period.

RESULTS.—*Relation of moisture to fungus invasion.*—Seeds of a durum wheat, variety Mindum, grown at St. Paul were hand harvested, cleaned, immediately dried to a moisture content of 11.0 per cent, and kept until used. Portions were inoculated with 2 isolates of each of 4 species of the *A. glaucus* group, stored at a relative humidity of 71 per cent at 25°C (average moisture content, 13.2 per cent), and tested periodically for fungus invasion. *A. amstelodami* and *A. restrictus* invaded most of the seeds within 6 months, the results with *A. ruber* were somewhat erratic, and *A. repens* invaded the seed slowly at this moisture content (Table 1). Apparently, a moisture content in wheat of 13.0–13.5 per cent is near the lower limit that will permit even very slow invasion by *A. ruber* and *A. repens*, but is not the lower limit for invasion by *A. restrictus* and *A. amstelodami*. The noninoculated controls remained free of fungi for 7 months, but after 9.5 months approximately 50 per cent of them yielded storage fungi, principally *A. restrictus*. In tests such as these, it has proved difficult to maintain control portions of seed absolutely free of storage fungi for more than a few months if the seeds are stored at moisture contents of 13.5–18 per cent and at a temperature favorable for growth of these fungi.

TABLE 1.—*Percentage of seeds of Mindum wheat invaded by 4 species of the Aspergillus glaucus group during storage for 9 months at 71 per cent relative humidity and 25°C*

Inoculated with	Percentage of seeds invaded during indicated period of storage (months)				
	4	5	6	7	9
Control—no inoculum	0	0	0	0	48[a]
Aspergillus amstelodami	32	64	90	83	97
A. restrictus	22	72	78	95	98
A. ruber	50	35	60	42	50
A. repens	10	15	22	48	35

[a] Mainly *A. restrictus*.

A similar test was made, with the same wheat and the same fungi at a relative humidity of 76 per cent (average moisture content, 14.3–14.6 per cent). *A. repens* invaded seed with this moisture content much more rapidly than it did seed containing 13.2 per cent moisture, and after 2 months it was recovered from approximately twice as many seeds as was any of the other 3 fungi. The noninoculated controls remained free of storage fungi for 2 months, but after 4 months 100 per cent of them yielded a mixture of *A. amstelodami*, *A. repens*, *A. ruber*, and *A. restrictus* (Table 2).

Four varieties of hard red spring wheat and one of durum, all grown at St. Paul, hand harvested, cleaned, and immediately dried to a moisture content of 11.0 per cent and stored dry until used, were inoculated with 2 isolates of *A. restrictus* and stored at a relative humidity of 71 per cent. Their moisture contents ranged from 13.0 to 13.6 per cent when tested after storage for 4 months. *A. restrictus* sporulated sparsely on the germ end of a few seeds in all samples within 5 months and was recovered from 60–99 per cent of the seeds stored for 9.5 months (Table 3).

TABLE 2.—*Percentage of seeds of Mindum wheat invaded by 4 species of Aspergillus glaucus group during storage for 4 months at 75–76 per cent relative humidity and 25°C*

Inoculated with	Percentage of seeds invaded during indicated period of storage (months)			
	1	2	3	4
Control—no inoculum	0	0	70	100
Aspergillus amstelodami	23	55	96	100
A. restrictus	2	58	96	100
A. ruber	14	48	96	100
A. repens	6	93	98	100

[4] The species here listed as *A. restrictus* is in the *A. restrictus* series as described by Thom and Raper (10), but differs in several respects from the *A. restrictus* described by Smith (8). Additional data on isolates of the *A. restrictus* series from wheat will appear in a subsequent paper.

TABLE 3.—*Percentage of seeds of 5 varieties of wheat invaded by Aspergillus restrictus during storage for 9.5 months at 71 per cent relative humidity and 25°C*

Variety	Moisture content (per cent)	Percentage of seeds invaded during indicated period of storage (months)				
		4	5	6	7	9.5
Durum						
Mindum	13.2	18	62	80	94	92
	13.2	28	84	80	88	98
Hard Red Spring						
Unknown	13.0	22	84	94	92	98
Willett	13.4	10		46	64	76
Thatcher	13.4	6	34	42	58	60
Mida	13.6	76	90	discontinued		

These tests provide additional evidence that *A. restrictus* is able to invade wheat seeds rather extensively at moisture contents as much as 1 per cent below those now specified for the regular grades of these classes of wheat. This agrees with results obtained from studies of samples from commercial bins (2).

Effect on seed germination of invasion of wheat seeds by species of the A. glaucus group.—Seeds of a durum wheat, variety Mindum, of the same lot as that described above, were stored at a relative humidity of 80 per cent. The controls were treated with sodium hypochlorite as specified above and not inoculated; the others were inoculated with the 4 species of the *A. glaucus* group. Two isolates of each species were used for inoculum. The moisture contents of 2 replicates of each treatment were determined after storage for 1 month; they ranged from 15.4 to 16.3 per cent, with most falling between 15.6 and 15.9 per cent. Germination was tested at intervals of 1 month.

In this test, approximately 100 per cent of the seeds inoculated with the various fungi were invaded within 1 month (Table 4). Within 2–3 months, the fungi were fruiting heavily on the germs of all seeds (Fig. 1, A). At 4 months, approximately 20 per cent of the controls also had been invaded by 1 or more members of the *A. glaucus* group; these were discarded. Germination of all lots inoculated with the fungi was very low as compared with the noninvaded controls; *A. restrictus* and *A. ruber* reduced the germination considerably more during storage for 4 months than did *A. repens* or *A. amstelodami*. *A. restrictus* was limited almost entirely to the germ. Its appearance on these seeds after storage for 2 months is shown in Figure 1, B. When such seeds were immersed in sodium hypochlorite for 1 minute, rinsed, and cultured on malt agar containing 20 per cent sodium chloride, *A. restrictus* grew out from the germs almost exclusively (Fig. 1, C). The other species of storage fungi tested were not so strictly limited to the germ but consistently invaded the germs more heavily and more extensively than other portions of the seed.

TABLE 4.—*Germination of Mindum wheat, after storage for 1–4 months at 25°C and 80 per cent relative humidity, when free of storage fungi and when inoculated with storage fungi*

Inoculum	Percentage of seeds that germinated after indicated months of storage [a]			
	1	2	3	4
None (control)	89	86	88	82
Aspergillus amstelodami	64	56	48	41
A. restrictus	66	44	39	20
A. ruber	74	60	49	15
A. repens	83	74	60	36

[a] Each figure is a mean of 2–6 replicates of 100 seeds each.

It has been suggested that even so light a treatment with sodium hypochlorite as was used in these tests might so alter the seeds as to make them more susceptible to subsequent invasion by storage fungi. To test this, seeds of Mindum, with and without immersion in sodium hypochlorite and with and without inoculation with a mixture of the 4 species of the *A. glaucus* group, were stored for 4 months at 80 per cent relative humidity and at 25°C. The moisture contents averaged 16.0 per cent, with a range of plus or minus 0.2 per cent. After storage for 1 month, 100 per cent of all inoculated seeds, whether or not treated with sodium hypochlorite, yielded storage fungi; after 4 months, approximately 25 per cent of the treated and noninoculated control yielded storage fungi. The data on germination are given in Table 5. Both the inoculated and noninoculated seeds that were not treated with sodium hypochlorite yielded approximately the same numbers of isolates of the 4 species of the *A. glaucus* group as did those treated and inoculated with the mixture. There was no evidence that treatment with sodium hypochlorite increased susceptibility of the seeds to invasion by these fungi.

DISCUSSION.—The 4 species of the *A. glaucus* group used in these tests rapidly invaded the germs of wheat stored at moisture contents of 15–16 per cent at a

TABLE 5.—*Germination percentages of Mindum wheat treated with 1 per cent sodium hypochlorite for 1 minute and those of wheat not so treated, each either not inoculated or inoculated with a mixture of 4 species of the Aspergillus glaucus group and stored for 1–4 months at 25°C and 80 per cent relative humidity*

Treatment	Percentage of seeds that germinated after indicated months of storage [a]			
	1	2	3	4
1 Per cent sodium hypochlorite, 1 minute				
Not inoculated [b]	89	86	88	82
Inoculated	53	52	38	30
No sodium hypochlorite				
Not inoculated [c]	46	48	31	30
Inoculated	48	42	31	22

[a] Each figure represents the mean of 2–7 replicates of 100 seeds each.

[b] Same samples as served for controls listed in Table 4.

[c] All seeds in this group were invaded by species of *A. glaucus*, apparently from inoculum naturally present.

FIG. 1. Members of the *Aspergillus glaucus* group fruiting on the germs of wheat seeds stored 2 months at a relative humidity of 80 per cent. A) A mixture of species fruiting externally on the germ end of the seeds. B) *A. restrictus* fruiting on the germ surface but not externally. C) A seed invaded by *A. restrictus* during storage; the seed was immersed in 1 per cent sodium hypochlorite for 1 minute, rinsed, and cultured on malt agar containing 20 per cent sodium chloride. *A. restrictus* was fruiting heavily and exclusively on the germ.

temperature of 25°C. This invasion was accompanied by a moderate to drastic reduction in germination percentage of the seed, as compared with the noninoculated controls. The germs were invaded more quickly and usually more heavily than other portions of the seeds, and invasion by *A. restrictus* was limited almost entirely to the germs. *A. restrictus* was found to be the predominant storage fungus in most of 27 samples of wheat, containing various amounts of sick seeds, from commercial bins (3), and it since has been found to be prevalent in additional samples of sick wheat from commercial bins. This, plus the evidence here presented, would seem sufficient to incriminate this fungus as a common cause of germ-damaged or sick wheat.

At moisture contents below 14 per cent, invasion of the seed by *A. restrictus* and *A. amstelodami* was more rapid than by *A. repens* and *A. ruber*. No evidence was obtained in the present tests of an effect of such invasion on germination and on development of discolored germs at these lower moisture contents, but it seems a fair presumption that extensive invasion of germs of wheat seed at a moisture content of 13.5–14.0 per cent eventually would result in the same sort of damage as in wheat at a moisture content of 14.5–15.0 per cent, even though a longer time might be required for such damage to appear. Wheat often is stored for 2–3 years. For such long storage periods, the safe moisture content limit would appear to be near 13.0 per cent, rather than at 14.0–14.5 per cent, as specified at present. In large bulks of stored grains, the moisture content often varies considerably from place to place in a given bin (4). For safe storage it is not sufficient that the average moisture content be 13.0 per cent; rather, none of the grain should have a moisture content in excess of 13 per cent for a long enough time to permit extensive development of those species of fungi now proved to invade and kill the germs.

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LITERATURE CITED

1. American Association of Cereal Chemists. 1956. Cereal laboratory methods. Ed. 6. Published by the Association, St. Paul, Minnesota.
2. Christensen, C. M. 1955. Grain storage studies XVIII. Mold invasion of wheat stored for sixteen months at moisture contents below 15 percent. Cereal Chem. **32**: 107-116.
3. Christensen, C. M. 1955. Grain storage studies XXI. Viability and moldiness of commercial wheat in relation to the incidence of germ damage. Cereal Chem. **32**: 507-518.
4. Christensen, C. M., and R. F. Drescher. 1954. Grain storage studies XIV. Changes in moisture content, germination percentage, and moldiness of wheat samples stored in different portions of bulk wheat in commercial bins. Cereal Chem. **31**: 206-216.
5. Coleman, D. A., and H. C. Fellows. 1925. Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. Cereal Chem. **2**: 275-287.
6. Hodgman, C. D. 1942. Handbook of chemistry and physics. Chemical Rubber Publ. Co., Cleveland, Ohio.
7. Kelley, D. F., B. M. Stahl, S. C. Salmon, and R. H. Black. 1942. Wheat storage in experimental farm-type bins. U. S. Dept. Agr. Circ. 637.
8. Smith, G. 1928. The identification of fungi causing mildew in cotton goods. The genus Aspergillus—Part II. Textile Inst. J. **22**: T110-T116.
9. Sorger-Domenigg, H., L. S. Cuendet, C. M. Christensen, and W. F. Geddes. 1955. Grain storage studies XVII. Effect of mold growth during temporary exposure of wheat to high moisture contents upon the development of germ damage and other indices of deterioration during subsequent storage. Cereal Chem. **32**: 270-285.
10. Thom, C., and K. B. Raper. 1945. A manual of the Aspergilli. Williams and Wilkins, Baltimore. 373 p.
11. U. S. Department of Agriculture, Production and Marketing Administration, Grain Branch. 1949. Handbook of official grain standards of the United States. Govt. Printing Office, Washington. 101 p.
12. Wink, W. A., and G. R. Spears. 1950. Instrumentation studies LVII. Equilibrium relative humidities above saturated salt solutions at various temperatures. Tappi **33**: 96-99.

ANALYSIS OF FUSARIUM RESISTANCE IN TOMATO BY GRAFTING EXPERIMENTS [1]

R. P. Scheffer

SUMMARY

Tomato plants were approach grafted in pairs, and tops were inoculated through scion spurs with bud-cell suspensions of *Fusarium oxysporum* f. *lycopersici*. All stock-scion combinations of resistant and susceptible plants were used. Resistance or susceptibility of plant tops (scions) was not changed by the resistance or susceptibility level of roots (stocks). Comparison of several types of top inoculations indicated that such methods usually fail because resistance is a characteristic of parenchyma of susceptible as well as of that of resistant plants. Conclusions were that 1) neither resistance nor susceptibility depend on root-produced factors that govern top reactions and that 2) resistance is a feature of the whole stem as well as of the roots.

The tomato inoculation experiments of Scheffer and Walker (4) and the evidence of Snyder et al (5) led to the conclusion that resistance to Fusarium wilt is found throughout stems of resistant plants. Scheffer and Walker inoculated tomato cuttings by allowing them to take in bud-cell suspensions by transpirational pull. When rooted, such cuttings from susceptible plants developed typical symptoms in due time, even though roots were not invaded by the pathogen. Cuttings from resistant plants treated likewise did not develop disease, showing that resistance is not localized in roots as other evidence would indicate (1).

Susceptibility rather than resistance may depend on a factor, or factors, produced in roots and transported to tops. In such case, resistant plants would be those that lacked the factor for susceptibility, making the fungus unable to develop or making the host insensitive to fungus products. The data of Waggoner and Dimond (6) from irradiation experiments could be interpreted in this way. The cutting inoculation experiments of Scheffer and Walker (4) do not eliminate this possibility, because roots were formed on cuttings before symptoms developed. If this theory is true, the experimental approach to a study of resistance and susceptibility is simplified, because these characters can be associated with definite factors.

Keyworth modified earlier views of localization of resistance and considered all above-ground stems to be resistant (2, 3). In susceptible plants, the pathogen was said to increase in roots enough to overcome the inherent resistance of stems. The view is supported by the often-observed difficulty of inoculating plant tops with vascular Fusaria.

The experiments described here were designed to determine whether or not resistance depends on lack of root-produced factors. Results also further clarify the question of location of resistance and possibly explain the difficulty of top inoculations.

MATERIALS AND METHODS.—The tomato (*Lycopersicon esculentum* Mill.) varieties Bonny Best and Jefferson were used as the susceptible and highly resistant hosts, respectively. Plants 4 or 5 in. high were approach grafted (Fig. 1), with Sealtex rubber bandage used to bind the grafts. Six to 10 days later, after graft unions were firm, plants were trimmed to give scion and stock combinations as follows: 1) susceptible scion on resistant stock, 2) susceptible scion on susceptible stock, 3) resistant scion on susceptible stock, and 4) resistant scion on resistant stock. The combination plants were then inoculated by placing the cut spur of the scion in a bud-cell suspension, (Fig. 2), thereby allowing transpirational pull to take in Fusarium cells. The inoculation procedure was essentially the same as that used in earlier work (4), except that inoculum was introduced through spurs of grafted plants rather than through bases of cuttings. Inoculum was prepared from shake cultures of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans., strain R5-6, by filtering and washing by centrifugation. Following inoculation, plants were kept in

FIG. 1. Method of grafting tomato plants. After union was firm, cuts were made at points 1 and 2, and inoculum was introduced through stub A. In some cases, cut 2 was made after inoculation.

[1] Accepted for publication January 4, 1957.
Michigan Agricultural Experiment Station Journal Article No. 1994.

the greenhouse under conditions favoring disease. Plants were observed closely for symptoms, and final data (vascular discoloration and wilt) were taken 28–35 days after inoculation. Results were expressed as the degree of vascular discoloration rather than as wilt, because transpirational conditions at the moment when readings are taken affect wilt but not discoloration.

Results.—In 3 separate grafting experiments (Table 1), 49 of 69 (or 71 per cent) plants with susceptible scions and resistant stocks developed typical symptoms. This does not differ materially from the 35 of 53 (or 66 per cent) of the susceptible scions on susceptible stocks that became diseased. Resistant scions did not show typical symptoms, regardless of the stock type; however, 13 plants with resistant scions on susceptible stocks and 1 plant with a resistant scion on a resistant stock had a trace of vascular discoloration. Some of these plants occasionally had flaccidity of top leaves under conditions favoring high transpiration, but leaf yellowing, epinasty, and other symptoms were absent. In every case where a plant with resistant scion and susceptible stock behaved in this way, Fusarium was shown by isolations to have invaded the susceptible stock and was present in the hypocotyl. Vascular dysfunction in every case apparently was at the graft union or below, since scions promptly recovered when they were removed from the plant and placed in water.

The plants in experiment 2 (Table 1) were older and harder than usual when inoculated, which may account for their low disease incidence. Their nutritional condition probably was such that severe disease should not be expected.

Disease developed more slowly in grafted plants than might be expected in plants inoculated by roots or by cuttings. A possible explanation is that only half the bundles in each stem took up inoculum, because the other half was the "tongue" of the graft, which connected with the noninoculated stock (Fig. 1). "Half plant" inoculation was studied further by comparing disease development in notched and unnotched Bonny Best cuttings, with inoculum introduced through the basal end. In notched cuttings, a wedge of stem was removed half way through the stem above the level of inoculum. The cuttings were rooted and in due time examined for disease development. Bundles immediately above the notches were not discolored and remained free of Fusarium as determined by isolations. Furthermore, disease developed somewhat more slowly in notched plants than in unnotched ones. Apparently the fungus did not pass readily from trace to trace in the tops of the plants.

Table 1.—*Relative vascular browning (VB) in grafted scions of 4 scion-stock combinations, with Bonny Best as the susceptible variety and Jefferson as the resistant variety*

Expt. No.	Scion-stock	Total no. of plants	No. with VB	Average VB rating [a]
1	Susceptible-resistant	17	16	
	Resistant-susceptible	14	0	
2	Susceptible-resistant	27	9	3.1
	Susceptible-susceptible	27	9	3.1
	Resistant-susceptible	32	2	trace
	Resistant-resistant	32	0	0
3	Susceptible-resistant	25	24	2.9
	Susceptible-susceptible	26	26	4.7
	Resistant-susceptible	28	11[b]	trace
	Resistant-resistant	26	1	trace

[a] VB rating = No. of major bundles in stem with browning times relative length of browning on a scale of 0 to 4. Plants without VB are not included in the average.

[b] Fusarium had invaded the stock and was present in the hypocotyl in every case.

Fig. 2. Method of inoculation with bud-cell suspension.

Previous experience indicated that little or no disease will develop from certain types of local top inoculations of intact plants. To obtain exact data, 4 groups of 15 plants each were inoculated by different methods and disease development compared. The first group was inoculated by dipping a small piece of cotton on a needle in a heavy spore suspension and forcing it into the stem; the cotton was left in the wound. Two of these plants developed a little vascular browning in 1 trace each. A second group was inoculated by placing a drop of spore suspension in a leaf axil and wounding the stem below the drop by means of a needle. No plants in this group developed symp-

toms. A third group was inoculated by cutting off a petiole from each plant about 3 in. from the stem and placing the attached stub in a dilute spore suspension. These plants all remained healthy. A fourth group was inoculated by the standard root-dip method; all succumbed with typical Fusarium wilt. In a similar experiment, 15 plants were top inoculated with cotton on a needle, 15 were root dipped, and 15 were inoculated as cuttings with bud cells. No top-inoculated plants became diseased, whereas the root- and cutting-inoculated plants all developed symptoms. Success of top inoculation of intact plants certainly will vary greatly, depending in part on the age and condition of plants, but obviously it was more difficult to obtain disease by such methods than by root inoculation.

Low inoculum levels might account for the low disease incidence resulting from top inoculations; therefore, spore suspensions of known concentrations were prepared by use of a hemacytometer, and cuttings were allowed to take up measured volumes of the suspensions. The number of Fusarium cells taken in was conservatively assumed to be in proportion to the volume of fluid uptake. Four levels of inoculum were compared, with 15 cuttings used at each level. The average inoculum for the 4 levels was 1520, 12,954, 130,050, and 1,203,600 spores per cutting. Following inoculation, cuttings were rooted and kept under conditions favoring disease. All plants in the experiment soon showed severe symptoms, indicating that Fusarium wilt will develop from a very low inoculum potential provided a high proportion of the vessels in all bundles is invaded.

Discussion.—Natural resistance of cortex and other parenchyma tissue in all varieties may account for the difficulty of top inoculation with vascular Fusaria. Such resistance is expected because these fungi are confined to vessels until late stages of disease; if parenchyma resistance were not present, a general stem rot would be expected. Few vessels probably received inoculum when stems were inoculated locally, since the vessels occupy a very small proportion of stem tissue in young plants. At best, a few vessels in 1 or 2 traces may be affected, with most inoculum left in parenchyma. The same or similar resistance may be present in root parenchyma as well. According to the best available evidence, vascular Fusaria enter roots mainly through root tips, possibly through wounds, and do not cause general rot until late stages of infection. Even root hair entrance is questionable. Parenchyma resistance may be a property of all tomato varieties; it probably is distinct from true disease resistance associated with certain varieties.

The above reasoning does not agree entirely with Keyworth's conclusion that resistance is a property of all stems (3). In experiments with Verticillium wilt of hop, tops inoculated with small doses of spores did not become diseased, whereas both resistant and susceptible plants were affected equally when massive doses were used (2). In contrast, in our experiments with Fusarium wilt of tomato, inoculation with very small doses of spores resulted in typical disease when inoculum was taken in through the majority of bundles, as was the case when cuttings were inoculated. A more massive dose applied locally, which would reach only a few vessels, gave little or no disease. Again, this suggests resistance in cortex or other parenchyma tissue, although Fusarium wilt of tomato and Verticillium wilt of hop certainly may differ in this respect.

The idea of a root-produced factor responsible for susceptibility may be supported by results of irradiation with X rays. Root irradiation before inoculation increased resistance, whereas shoot irradiation decreased or did not change resistance (6). An interpretation of this might be that root irradiation destroyed the ability of roots to produce the susceptibility factor, thus plants were resistant; however, irradiation experiments were concerned with relative resistance of inherently susceptible plants, whereas the present experiments were concerned with the difference between susceptible plants and plants with inherent resistance of a very high order. We cannot assume that the manipulatable resistance of inherently susceptible plants is similar to so-called true resistance.

Grafting experiments described here differ from earlier ones chiefly in the point of inoculation. Previously, all plants in grafting experiments were root inoculated (1). If roots were susceptible, plants became diseased regardless of the level of scion resistance, since roots obviously must support tops. The difficulty of direct stem inoculation was overcome here by allowing spurs of the scions to take in bud cells by transpirational pull. Scion inoculation by-passed the root system, apparently because Fusarium did not grow readily through parenchyma of either resistant or susceptible plants. Inoculated stem traces were in effect isolated from the fungus-free traces that led to the stock. Results of scion inoculation did not agree with results of other experiments where roots were inoculated (1), and different conclusions were drawn accordingly.

Resistance or susceptibility of the scion, which is stem tissue, was not altered by the character of the stock. Resistant tops (scions) behaved like resistant plants, whether the root (stock) was resistant or susceptible, and susceptible tops were susceptible regardless of the root type. There was no evidence of a root-produced factor responsible for susceptibility or resistance. On the positive side, observations confirm earlier conclusions based on cutting inoculation experiments (4), that true resistance to Fusarium wilt is not localized in the root system but is a characteristic of the stem as well. It is not clear whether or not factors produced throughout the plant are responsible for susceptibility or resistance.

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LITERATURE CITED

1. Heinze, P. H., and C. F. Andrus. 1945. Apparent localization of Fusarium resistance in the Pan America tomato. Am. J. Botany **32**: 62-66.
2. Keyworth, W. G. 1953. Verticillium wilt of the hop. VI. The relative roles of root and stem in the determination of wilt severity. Ann. Appl. Biol. **40**: 344-361.
3. Keyworth, W. G. 1955. Studies on the mechanism of invasion of plants by vascular pathogens. In Mechanisms of microbial pathogenicity. Fifth Symposium of the Society for General Microbiology. Cambridge University Press. p. 320-333.
4. Scheffer, R. P., and J. C. Walker. 1954. Distribution and nature of Fusarium resistance in the tomato plant. Phytopathology **44**: 94-101.
5. Snyder, W. C., K. F. Baker, and H. N. Hansen. 1946. Interpretation of resistance to Fusarium wilt in tomato. Science **103**: 707-708.
6. Waggoner, P. E., and A. E. Dimond. 1956. Altering disease resistance with ionizing radiation. Phytopathology **46**: 125-127.

THE EFFECTS OF SOME LEAF BLIGHTS OF CORN ON GRAIN YIELD[1]

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SUMMARY

The effects on grain yield of artificially induced epiphytotics of northern corn leaf blight, of southern corn leaf blight, and of Helminthosporium leaf spot in the field were determined.

The severity of the 3 diseases appeared to be determined not by the primary infection induced by artificial inoculation but rather by the development of secondary infection, which was influenced by weather conditions.

In an experiment where a severe epiphytotic of northern corn leaf blight became established, the most resistant hybrid yielded over 70 bushels/acre in excess of that of the most susceptible hybrid. Where double-cross hybrids with different numbers of resistant inbred lines in their pedigrees were grown in the presence of a severe epiphytotic, each resistant inbred line substituted into the double-cross hybrids resulted in an average yield increase of about 15 bushels/acre. In experiments conducted during the hot, dry growing season of 1952, when secondary infection failed to develop until corn was approaching maturity, the increase in yield following the substitution of resistant inbred lines into double-cross pedigrees was not significant statistically. From these experiments, a basis may be established for making reasonably accurate estimates of yield losses due to northern corn leaf blight. Early onset of the disease, 2–3 weeks after silking, may be expected to cause severe losses in grain yield. If the disease does not become conspicuous until mid-September or 6–8 weeks after silking, no appreciable reduction in yield is apt to occur.

In a limited number of experiments with southern corn leaf blight, the same general trends were observed. An average yield increase of about 5.4 bushels/acre was realized for each increment of resistance introduced into the pedigrees of susceptible double crosses.

In 1 experiment with Helminthosporium leaf spot, the completely resistant hybrid K61 × Pr1 produced about 94 bushels/acre in the presence of a severe epiphytotic of the disease, whereas the fully susceptible single cross K61 × Pr yielded only about 19 bushels/acre. In the absence of the disease, the performances of the 2 hybrids were about equal.

Comparatively little is known about the effect of most corn (*Zea mays* L.) diseases on grain yield. This is especially true of certain leaf blights that do not attack ears but may have an indirect influence on the amount of dry matter stored in the grain. Estimates are available on the losses attributable to leaf diseases of corn, but at best these are rough guesses not based on experimental data. Quantitative data would be of value in determining the real economic importance of a disease and might also be useful in predicting yields in years when epiphytotics appeared.

This paper reports results of a study to determine, under artificially induced epiphytotics, the effects on grain yield of northern corn leaf blight, of southern corn leaf blight, and of Helminthosporium leaf spot.

Northern corn leaf blight, incited by *Helminthosporium turcicum* Pass., is the most important of the 3 diseases. It is sporadic in occurrence; in the United

[1] Accepted for publication January 4, 1957.
Joint contribution from the Department of Botany and Plant Pathology, the Department of Agronomy, Purdue University, Lafayette, Indiana, and Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Journal Paper No. 1017 of the Purdue University Agricultural Experiment Station.

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States it is confined usually to the eastern half of the country. Occasionally, the disease may become very severe in localized areas. Moderate temperatures and frequent and heavy dews favor spread of the disease. The inheritance of resistance to this disease is determined by several genes[3].

Southern corn leaf blight, incited by *Helminthosporium maydis* Nisikado & Miyake, is found generally south of the Ohio River Valley and eastward to the Atlantic coast. The disease appears to thrive at somewhat higher temperatures than does northern corn leaf blight. The importance of the disease is not fully known, but in some years it is responsible for considerable leaf killing. Resistance to this disease, like that to northern corn leaf blight, is conditioned by multiple factors[4].

Helminthosporium leaf spot, incited by Race I of *Helminthosporium carbonum* Ullstrup, is found throughout the eastern half of the United States; its development and spread are favored by moderate temperatures and high humidity. This pathogen is the most virulent of the 3 considered here, but the disease is of negligible importance because few inbred lines are susceptible and because resistance is determined by a single dominant gene[5]. This disease was used in these studies only as a tool to determine the effect of a leaf-infecting fungus pathogen on grain yield.

MATERIALS AND METHODS.—All experiments were conducted under field conditions. Plots were 1 × 10 or 2 × 5 hills in size and were randomized in 4–8 replications. Hills were spaced 40 × 40 in. and thinned to a maximum of 3 plants. In all experiments, 2 complete blocks were planted, one of which was artificially inoculated; the other was left noninoculated. Both were planted on soil of about the same type and fertility level as far as could be ascertained by appearance, but they were separated by at least ¼ mile to avoid spread of disease from the inoculated to the noninoculated block. Plantings in both blocks were made on approximately the same date, as were the harvests in each. Yields were calculated in the usual manner on the basis of bushels per acre of shelled corn at 15.5 per cent moisture.

In experiments of the type reported here, where the differential effects of a disease on resistant and susceptible hybrids are to be ascertained, it is necessary to plant 2 blocks of the same materials in order to avoid confounding the determination of disease effects with heritable differences in yield potential between the hybrids compared. For example, the variance in yield between a resistant and a susceptible hybrid when grown only in an inoculated block would be composed of the differential effects of disease and also of the differential effects of inherent yield potential. These 2 components could not be separated under such circumstances. It should be pointed out, however, that if the hybrids to be compared were nearly isogenic and if resistance were determined by a single gene, as in the case of Helminthosporium leaf spot, then direct comparison between hybrids in only an inoculated block would be permissible. In experiments on northern corn leaf blight and on southern corn leaf blight, where in each case resistance is known to be determined by many genes, the hybrids did not approach being isogenic and differed widely in yield potential as well as in resistance. A noninoculated block allows for the evaluation of differences in heritable yield potential between any 2 hybrids compared. When 2 blocks are planted, the confounding factor of differences in soil fertility is introduced. This effect of soil fertility is separated from disease effects by the presence of resistant hybrids in both blocks to serve as controls. Implicit in the choice of the experimental design and calculation, is the assumption, based on preliminary observations, that resistant hybrids are less affected by disease than are susceptible hybrids, and that differences in soil fertility between the inoculated and noninoculated blocks affect resistant and susceptible hybrids alike.

To obviate the confounding factors and to determine only the effect of a disease on grain production, the yields of a resistant hybrid when inoculated and when noninoculated were compared with the yields of a less resistant hybrid under the same treatments. This was calculated as follows: (Yield of noninoculated resistant hybrid minus yield of inoculated resistant hybrid) minus (Yield of noninoculated susceptible hybrid minus yield of inoculated susceptible hybrid). As an example, the data in Table 1 for A × Tr and Mo21a × Ky114 would be used in the calculation as follows: $(113.3 - 111.2) - (110.4 - 34.8) = -73.5$. This value, − 73.5 bushels/acre, is a measure of how much more the yield of the susceptible hybrid was affected by the disease than was the yield of the

TABLE 1.—*Mean yields*[a] *in bushels per acre of resistant and susceptible single-cross hybrids when inoculated with Helminthosporium turcicum and when not so inoculated*

Hybrid	Noninoculated	Inoculated
Susceptible hybrids		
A × Tr	110.4	34.8
187-2 × 540	121.1	46.4
K64 × T14	122.5	64.8
38-11 × Mo940	150.6	89.0
Resistant hybrids		
CI.14 × A71	101.2	82.7
Ky36-11 × L317	114.5	100.8
K230 × T528	98.2	94.8
Mo21a × Ky114	113.3	111.2

[a] LSD = 11.2 bushels per acre at the 5 per cent level; 14.8 bushels per acre at the 1 per cent level.

[3] Jenkins, M. T., and Alice L. Robert. 1952. Inheritance of resistance to the leaf blight of corn caused by Helminthosporium turcicum. Agron. J. 44: 136-140.

[4] Pate, J. B., and P. H. Harvey. 1954. Studies on the inheritance of resistance in corn to Helminthosporium maydis leaf spot. Agron. J. 46: 442-445.

[5] Ullstrup, A. J., and A. M. Brunson. 1947. Linkage relationships of a gene in corn determining susceptibility to a Helminthosporium leaf spot. J. Am. Soc. Agron. 39: 606-609.

resistant hybrid. Conversely, + 73.5 bushels/acre, would represent how much less the yield of the resistant hybrid was affected by the disease than was the yield of the susceptible hybrid. The calculation takes into consideration and corrects for any differences, such as soil fertility, between the inoculated and noninoculated blocks that would influence yield, as well as for differences in genetically determined yielding ability of the 2 hybrids being compared. It does not take into account possible interaction between hybrids and soil fertility levels. This, if present, was considered to be of a magnitude insufficient to vitiate the measure of disease effects.

Resistant and susceptible single- and double-cross hybrids were used in the experiments with northern corn leaf blight and in those with southern corn leaf blight. In the experiment with Helminthosporium leaf spot, only 1 resistant and 1 susceptible single cross were used. The effects of different levels of resistance on disease severity and grain yield were determined by using double crosses that had 0, 1, 2, 3, or 4 resistant inbred lines in their pedigrees.

Inocula were prepared by growing the respective fungi on potato-dextrose agar in petri dishes for about 3 weeks at 22°–25°C. Each culture (and its agar substrate) was ground in water in a Waring Blendor and the suspension of spores and hyphal fragments filtered through a single layer of cheesecloth. The suspensions were taken to the field and further diluted with water before application.

The method of inoculation with each of the 3 pathogens was essentially similar to that originally described by Elliott and Jenkins[6]; the suspension of spores and hyphal fragments was introduced into the whorls of the plants by means of a compressed-air sprayer. The number of inoculations varied from 2 to 6 in different years and in different experiments. The first was applied when plants were 18–24 in. in height, or about the third or fourth week of June, and inoculations were continued at 5–7-day intervals. The concentration of inoculum was not constant for every experiment. Concentrated inoculum was used when the number of inoculations were few and dilute when 6 inoculations were made. The essential objective was to establish primary infection uniformly over the entire block.

Results.—*Northern corn leaf blight.*—In experiments performed in 1948, resistant and susceptible single crosses were inoculated as described. Weather conditions were extremely favorable so that by the latter half of August susceptible hybrids were severely affected; they were rated 4–4.5 on a scale of 1 to 5 in which progressively higher ratings represented increasing disease severity. In the disease-free plots, the susceptible hybrids in general yielded more than did the resistant hybrids (Table 1). In contrast, yields in the presence of the disease were much higher in the resistant than in the susceptible hybrids. That this resulted from a greater effect of the disease on the susceptible than on the resistant hybrids is shown by the data in Table 2.

Table 2.—*Calculated data, in bushels per acre, representing the superiority of yields of resistant hybrids over those of susceptible hybrids when subjected to a severe epiphytotic of northern corn leaf blight*[a]

Susceptible hybrid	Data obtained when yield of indicated resistant hybrid was compared with yields of different susceptible hybrids[b]			
	CI.14 × A71	Ky36-11 × L317	K230 × T528	Mo21a × Ky114
A × Tr	+ 57.1	+ 61.9	+ 72.2	+ 73.5
187-2 × 540	+ 56.2	+ 61.0	+ 71.3	+ 72.6
K64 × T14	+ 39.2	+ 44.0	+ 54.3	+ 55.6
38-11 × Mo940	+ 43.1	+ 47.9	+ 58.2	+ 59.5
Mean	+ 48.9	+ 53.7	+ 64.0	+ 65.3

[a] All values are statistically significant at the 1 per cent level.

[b] Data in Table 1 was used to make these calculations as follows: (Yield of noninoculated resistant hybrid minus yield of inoculated resistant hybrid) minus (Yield of noninoculated susceptible hybrid minus yield of inoculated susceptible hybrid).

It should be pointed out that the 2 single crosses CI.14 × A71 and Ky 36-11 × L317 are only partially resistant. The inbred line A71, although one of the most resistant in its maturity class, does not possess the high resistance of certain other inbred lines. L317 is susceptible and would tend to lower resistance of the single cross when combined with Ky36-11.

In 1950, a similar experiment was performed with a different group of resistant and susceptible single crosses. Plantings in the experiment were arranged so that each test plot was adjacent to a row of the very susceptible hybrid A × Tr. Inoculum was applied only to these "spreader rows" and not to the test plots. Infection of the latter originated from secondary inoculum developed naturally in the primary lesions produced on the "spreader rows." By this method, inoculation of plants in the test plots more closely simulated that occurring in a natural epiphytotic than did that in the previous experiment. By late August, the susceptible hybrids in the test plots showed moderate to severe infection. Again, the yields of resistant hybrids were much less affected by the disease than were those of susceptible hybrids (Table 3).

If double-cross hybrids are used, it is possible to have 5 levels of resistance by varying, from 0 to 4, the number of resistant inbred lines in a given pedigree. To determine the effect of different levels of resistance on the severity of northern corn leaf blight and on grain yield, 15 double-cross hybrids were made up such that there were 3 hybrids of different pedigrees at each of the 5 levels of resistance. The pedigrees of these hybrids are as follows (resistant inbred lines are indicated in italics): 1 = (*K175* × *Ky36-11*) × (*R39* × *CI15*); 2 = (Wf9 × *R39*) × (*K175* × *K148*); 3 = (Wf9 × W22) × (*K175* ×

[6] Elliott, Charlotte, and M. T. Jenkins. 1946. Helminthosporium leaf blight of corn. Phytopathology 36: 660-666.

TABLE 3.—*Calculated data, in bushels per acre, showing the superiority in yields of resistant hybrids over the yields of susceptible hybrids in the presence of a moderately severe epiphytotic of northern corn leaf blight*[a]

Susceptible hybrid	Data obtained when yield of indicated resistant hybrid was compared with yields of different susceptible hybrids[b]			
	CI.15 × R39	K148 × K175	CI.23 × NC34	T105B × NC34
P8 × CI.7	+ 21.1	+ 23.2	+ 31.0	+ 33.9
P8 × Mo940	+ 35.2	+ 37.3	+ 45.1	+ 48.0
R30 × H21	+ 29.1	+ 31.2	+ 39.0	+ 41.9
T14 × K64	+ 25.7	+ 27.8	+ 35.6	+ 38.5
Mean	+ 27.8	+ 29.9	+ 35.7	+ 40.6

[a] All values are statistically significant at the 1 per cent level.
[b] Calculated as indicated in Table 2.

Ky36-11); 4 = (Wf9 × W22) × (P8 × *R39*); 5 = (P8 × W22) × (38-11 × 187-2); 6 = (*R39* × *CI15*) × (*K148* × *Ky36-11*); 7 = (W22 × *Ky36-11*) × (*K175* × *R39*); 8 = (Wf9 × P8) × (*R39* × *C115*); 9 = (Wf9 × P8) × (W22 × *Ky36-11*); 10 = (Wf9 × P8) × (W22 × 187-2); 11 = (*R39* × *CI15*) × (*K148* × *K175*); 12 = (P8 × *R39*) × (*K175* × *Ky36-11*); 13 = (P8 × W22) × (*K148* × *Ky36-11*); 14 = (P8 × W22) × (Wf9 × *K148*); 15 = (P8 × Wf9) × (K159 × Mo940).

Following inoculation, the moderate temperatures and heavy dews prevailing during the growing season of 1951 favored secondary spread of the disease so that by August 20 a severe epiphytotic was established. There was some disease in the noninoculated block; on September 18, the most susceptible hybrids had a rating of 2 on the scale of 1 to 5 referred to above. The results of this experiment are summarized in Table 4. The effect on yield of each stepwise addition of resistance in the pedigrees was determined by calculating the "difference between differences" as illustrated above. As an example, 9 comparisons could be made between the most resistant hybrids (hybrids 1, 6, and 11) and the most susceptible hybrids (hybrids 5, 10, and 15). The mean increase in yield of the fully resistant hybrids over the completely susceptible hybrids as determined by the 9 comparisons was 61.3 bushels per acre. The mean effect on yield of each successive increment of resistance was remarkably uniform and averaged 15.3 bushels per acre.

Further experiments were continued in subsequent years on the effect of different levels of resistance to northern corn leaf blight on yield. Essentially the same procedures as described above were used. In these studies, the susceptible double-cross hybrids U. S. 13, which is (Wf9 × 38-11) × (Hy × L317), and Indiana 844, which is (Wf9 × 38-11) × (Hy × Tr), were used. The inbred lines used to substitute into these pedigrees were resistant derivatives of the respective standard inbred lines and had been "recovered" in the course of a corn-disease-resistance breeding project at Purdue University. These "recovered" inbred lines

TABLE 4.—*Summary of 1951 data for the effect of northern corn leaf blight on grain yields*

Differences in no. of resistant inbred lines in double-cross hybrids	1	2	3	4
No. of comparisons made (specific comparisons indicated in brackets)[a]	36 [0 *vs* 1, 1 *vs* 2, 2 *vs* 3, 3 *vs* 4]	27 [0 *vs* 2, 1 *vs* 3, 2 *vs* 4]	18 [9 *vs* 3, 1 *vs* 4]	9 [0 *vs* 4]
Increases in yield attributable to added resistance (bushels/acre)[b]	15.6	30.6	45.8	61.3
Yield increments for each additional increment of resistance (bushels/acre)	15.6	15.0	15.2	15.5

[a] Specific comparisons designated by number of resistant inbred lines in the double-cross hybrids; thus, 0 *vs* 4 indicates that hybrids in which there were no resistant inbred lines were compared with hybrids in which all 4 of the component inbred lines were resistant.
[b] Method of calculating yield increases due to resistance is given in Table 2.

were appreciably higher in resistance than were the respective inbred lines (recurrent parents) from which they originated. The prefix "R" was used to designate the respective "recovered" inbred lines. Twelve different variations in the pedigree of Indiana 813 were made up thus: (Wf9 × 38-11) × Hy × L317); (RWf9 × 38-11) × (Hy × L317); (Wf9 × R38-11) × (Hy × L317); (Wf9 × 38-11) × (RHy × L317); (Wf9 × 38-11) × (Hy × RL317); (RWf9 × R38-11) × (Hy × L317); (Wf9 × 38-11) × (RHy × RL317); (RWf9 × 38-11) × (RHy × RL317); (Wf9 × R38-11) × (RHy × RL317); (RWf9 × R38-11) × (RHy × L317); (RWf9 × R38-11) × (Hy × RL317); and (RWf9 × R38-11) × (RHy × RL317). The same scheme was followed in making up 12 variations in the pedigree of Indiana 844. In 1 experiment in 1954, the resistant inbred lines used as replacements for the standard susceptible inbred components in the pedigree of Indiana 844 were Ky36-11, CI.15, R39, and K148.

A summary of results of these experiments is shown in Table 5. The hot, dry weather during most of the 1952 growing season delayed the secondary spread of the pathogen until mid-September so that the epiphytotic was unusually mild. This low level of disease development is reflected in the negligible difference in yield between the resistant and susceptible hybrids and in the nonsignificant regression coefficients in that year. The epiphytotics established in 1954 and in 1955 were of moderate severity, and this is shown in the relative magnitude of increases in grain yield resulting when resistant inbred lines were substituted in the pedigrees of the double crosses.

Southern corn leaf blight.—Experiments on the effect of southern corn leaf blight were conducted in the same manner as those described for northern

TABLE 5.—*Summary of results of experiments to determine the effect on grain yield of different levels of resistance in double-cross hybrids to northern corn leaf blight and to southern corn leaf blight*

Disease[a]	Year	Hybrid designation	Total yield increases, in bushels per acre, resulting from substituting indicated number of resistant inbred lines in double-cross pedigrees				Yield increments, in bushels per acre, due to indicated stepwise increase in no. of resistant inbred lines in pedigree				Regression coefficient (bushels per acre per resistant inbred line substituted in double crosses)
			1	2	3	4	0 to 1	1 to 2	2 to 3	3 to 4	
NCLB	1951	[b]	15.6	30.6	45.8	61.3	15.6	15.0	15.2	15.5	15.3*[c]
	1952	Ind. 844	1.6	3.5	4.9	6.2	1.6	1.9	1.4	1.3	1.7
		U.S. 13	1.3	1.3	3.9	7.3	1.0	0.0	2.6	3.4	1.1
	1954	Ind. 844	5.6	15.3	16.7	16.4	5.6	9.7	1.4	−0.3	6.3*
		Ind. 844[d]	5.9	12.7	19.0	23.8	5.9	6.8	6.3	4.8	6.2*
		U.S. 13	4.8	11.8	14.4	16.1	4.8	7.0	2.6	1.7	5.2*
	1955	Ind. 844	6.9	15.6	22.2	28.5	6.9	8.7	6.5	6.3	7.5*
		U. S. 13	4.6	9.8	13.7	17.4	4.6	5.2	3.9	3.7	4.7*
SCLB	1955	[e]	4.9	11.3	17.4	19.7	4.9	6.4	6.1	2.3	5.4*

[a] NCLB = northern corn leaf blight; SCLB = southern corn leaf blight.
[b] These double crosses were not standard numbered hybrids. Their pedigrees are given in the text.
[c] Starred (*) regression coefficients indicate statistical significance beyond the 1 per cent level.
[d] The resistant inbred lines substituted in the pedigree of Ind. 844 in this experiment were CI.15, Ky36-11, R39, and K148.
[e] These double crosses were not standard numbered hybrids. Resistant and susceptible inbred components are given in the text.

corn leaf blight. In 1950, 5 resistant and 5 susceptible single crosses were inoculated; the same hybrids were grown in a noninoculated block. The yields of each resistant hybrid in the inoculated and in the noninoculated blocks were compared with the yields of susceptible hybrids subjected to the same treatments. The results (Table 6) show that in nearly all comparisons the resistant hybrids yielded significantly more in the presence of the disease than did the susceptible hybrids. A similar experiment, conducted in 1952, in which 3 resistant and 3 susceptible double crosses were used, showed the same general increase in yield of the resistant over the susceptible hybrids when grown under an epiphytotic (Table 7). In a third experiment, run in 1955, 15 double crosses were made up so as to have 0, 1, 2, 3, or 4 resistant inbred lines in their pedigrees. Three hybrids, each of a different pedigree, were represented at each of the 5 levels of resistance (0 to 4 resistant inbred lines in a given pedigree). The resistant inbred lines used to make up the double crosses were C103, Tr, MoG, Os426, M14, and W20. The susceptible inbred components were L289, L317, R61, Wf9, P8, and Pr1. The results of this experiment, summarized in Table 5, show that the fully resistant hybrids yielded an average of 19.7 bushels per acre more than did the fully susceptible hybrids. The regression coefficient of 5.4 bushels per acre reflects the mean increase in yield realized for each increment of resistance (resistant inbred line) substituted in the double-cross pedigrees.

Helminthosporium leaf spot.—To test the effect of Helminthosporium leaf spot on yield, the resistant single cross K61 × Pr1 and the susceptible single cross K61 × Pr were used. The inbred lines K61 and Pr are susceptible to infection by Race 1 of *H. carbonum*. Pr1 is a resistant recovery of Pr following an outcross to the resistant inbred Hy, with 6 subse-

TABLE 6.—*Calculated data, in bushels per acre, representing the superior yield of the resistant hybrids over the susceptible hybrids in the presence of an epiphytotic of southern corn leaf blight*

Susceptible hybrids	Data obtained when yield of indicated resistant hybrid was compared with yields of different susceptible hybrids[a]				
	Tr × M14	MoG × Os426	C103 × Os426	MoG × Tr	C103 × W20
Wf9 × R61	+ 16.0	+ 18.2	+ 18.2	+ 23.0	+ 13.3
L289 × P8	+ 12.5[b]	+ 14.7	+ 14.7	+ 20.0	+ 9.8[b]
L317 × Pr1	+ 25.3	+ 27.5	+ 27.5	+ 32.8	+ 22.6
P8 × R61	+ 15.0	+ 17.2	+ 17.2	+ 22.5	+ 12.3[b]
P8 × Pr1	+ 16.5	+ 18.7	+ 18.7	+ 23.5	+ 13.8
Mean	+ 17.1	+ 19.3	+ 19.3	+ 24.4	+ 14.4

[a] These data calculated as indicated in Table 2.
[b] All values are statistically significant at the 1 per cent level except 12.5 and 12.3, which are significant at the 5 per cent level, and 9.8, which is not statistically significant.

TABLE 7.—*Calculated data, in bushels per acre, showing the superior yield of resistant double crosses over the susceptible double crosses in the presence of southern corn leaf blight*

Susceptible double crosses	Data obtained when yield of indicated resistant double cross was compared with yields of different susceptible double crosses[a]: (MoG × C103) × (W20 × Tr)	(MoG × C103) × (Os426 × M14)	(W20 × Tr) × (Os426 × M14)
(L317 × L289) × (P8 × R61)	+ 28.8	+ 22.3	+ 20.9
(L317 × L289) × (Wf9 × Pr1)	+ 33.1	+ 26.6	+ 25.2
(P8 × R61) × (Wf9 × Pr1)	+ 32.1	+ 25.6	+ 24.2
Mean	+ 31.3	+ 24.8	+ 23.4

[a] All values are statistically significant at the 1 per cent level. The data were calculated as indicated in Table 2.

quent backcrosses to Pr. The 2 lines Pr and Pr1 are thus genetically quite similar except for the gene pair *hm/hm*, which confers susceptibility to Race I of *H. carbonum*. The inbred lines are indistinguishable in plant type and yield potential.

Replicated plantings of the 2 single crosses were made in each of 2 locations, which were separated by about ⅜ mile. One planting was inoculated when in the boot stage; the other was not artificially inoculated. Weather conditions favored development of the disease throughout the remainder of the growing season. The similarity in yield potential between the 2 hybrids is shown by their performance in the non-inoculated block (Table 8). The superior yield of the resistant hybrid in the presence of the disease over its yield in the absence of the disease is attributed to the higher level of soil fertility in the inoculated block. The influence of disease resistance on yield in the presence of the disease is shown by the calculated difference of 72.6 bushels per acre.

DISCUSSION.—Initial infection following artificial inoculation with a spore suspension of *H. turcicum* was established somewhat earlier in the life history of the host than is commonly observed under natural conditions in the east-central part of the Corn Belt. When moderate to severe natural epiphytotics of the disease occur, lesions generally are first seen shortly after silking and do not become abundant until the middle to the latter part of August or 3–4 weeks after fertilization. Earlier onset of the disease resulting from artificial inoculation might be expected to be more destructive than natural infection, which takes place later. Primary lesions following artificial inoculation are so few in number that it is unlikely that they do any appreciable damage to the host. This is borne out in experiments made in 1952, in which little, if any, secondary spread of the disease developed until mid-September. Under such circumstances, reductions in grain yield in the susceptible hybrids were not statistically significant. The greatest destruction of leaf tissue occurs when secondary spread of the disease becomes abundant. The time when this takes place in either natural or artificially induced epiphytotics depends on weather conditions.

TABLE 8.—*Actual yields of 2 single crosses in the absence of Helminthosporium leaf spot compared with their yields in the presence of the disease, and the calculated difference in yields due to resistance*

Single cross	Non-inoculated block	Inoculated block	Difference
	Bushels/acre	Bushels/acre	Bushels/acre
K61 × Pr1 (resistant)	84.4	93.7	+ 9.3
K61 × Pr (susceptible)	82.6	19.3	— 63.3
Difference due to resistance[a]			+ 72.6
LSD at 1 per cent level	3.8	6.8	

[a] Calculated as indicated in Table 2.

The high level of severity and early onset of northern corn leaf blight induced in the experiments in 1951 are rarely attained under natural conditions in Indiana and neighboring areas. Natural epiphytotics in this general area have been comparable in severity and in time of appearance to the artificial epiphytotics established in 1950, 1954, and 1955.

On the basis of these experiments, natural epiphytotics of northern corn leaf blight that become severe 2–3 weeks after fertilization may be expected to depress yields as much as 50 per cent. When the disease does not become severe until 4–5 weeks after fertilization, losses of 15–25 per cent may take place. If the onset of the disease is delayed until mid-September or 6–8 weeks after full silk, no significant losses in grain yield are likely to occur.

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OSMOTIC PRESSURE AND PLANT VIRUS LOCAL LESIONS[1]

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SUMMARY

When Pinto bean plants were placed in solutions of glucose, sucrose, or $CaCl_2$ and inoculated 2 days later with tobacco mosaic virus, fewest lesions developed on those plants in the solutions with the highest osmotic concentrations. In further tests with osmotic pressures of 0.0, 0.6, 1.3, and 2.0 atmospheres, fewer lesions occurred at 1.3 atmospheres than at 0.0 atmosphere when plants were placed in solutions of sucrose, glucose, fructose, maltose, or $CaCl_2$; at 0.6 and 2.0 atmospheres with solutions of fructose; and at 2.0 atmospheres with $CaCl_2$. In all cases, the greatest effect occurred at 1.3 atmospheres.

Although a greater number of lesions was found on plants placed in K_2SO_4 solutions of 0.6, 1.3, or 2.0 atmospheres than on plants placed in distilled water, the least number occurred at 1.3 atmospheres.

Time of inoculation in relation to exposure of plants to solutions of various osmotic pressures was found to affect lesion number; the effect of osmotic pressure on lesion number was greatest when plants were exposed to the solutions at least 2 days before inoculation, although this period varied with the different chemicals tested.

In indirect effect of osmotic pressure on the virus through an alteration of the physiology of the suscept was inferred, because of lack of statistically significant differences in lesion number when solutions of various osmotic pressures were mixed with the virus prior to inoculation.

Expressed fluids of leaf tissue of Pinto bean plants that had been placed in solutions of sucrose with osmotic pressures of 0.0, 0.6, and 1.3 atmospheres were 7.2, 8.6, and 11.7 atmospheres, respectively. The osmotic pressures of expressed fluids of leaf blades, petioles, and pulvini of soil-grown Pinto bean plants were 7.3, 8.9, and 10.7 atmospheres, respectively. When soil-grown plants were inoculated with tobacco mosaic virus, no lesions developed on the pulvini, few on the petioles, and many on the leaf blades.

When the stems of severed bean plants of varieties other than Pinto were placed in sucrose solutions of 0.6, 1.3 and 2.0 atmospheres and the leaves inoculated with tobacco mosaic virus, a higher number of lesions developed in most cases than on plants in distilled water.

The data suggest that osmostic pressure may account for variations in lesion number on plants inoculated with virus and held under various environmental conditions. Periods of darkness, exposure to light, time of day of inoculation, etc. could change the osmotic pressure of the plant fluids, thus affecting lesion number.

Since considerable work is now centered about virus inhibition due to various chemicals and plant extracts, there is a need for evidence as to the effects of the colligative properties of the solutions of the inhibitors with respect to virus inhibition. In most work with virus inhibitors, mineral salts, or sugars, the checks consist of distilled water. Therefore, in this work the effect of osmotic pressure on virus lesion number is especially considered.

LITERATURE REVIEW.—In search of the literature, no work could be found that related the effects of osmotic pressure to virus disease development, although there are many reports of differences in the number of local lesions induced by viruses on plants subjected to various environmental factors. For example, there are several reports of more lesions on various plants kept in the shade before inoculation than on plants kept in sunlight or under the usual greenhouse light conditions (1, 4, 6, 8, 12).

[1] Accepted for publication January 14, 1957.

Paper No. 597 Department of Botany and Plant Pathology, Ohio State University. Part of a thesis presented to the Graduate School of The Ohio State University in partial fulfillment of the requirements for the degree Doctor of Philosophy. The author is grateful to Dr. C. C. Allison, Dr. P. Allison, and Dr. G. Barnes for their helpful suggestions throughout the course of the investigation.

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According to the work of Tinsley (11), increasing the amount of water supplied to plants of *Nicotiana glutinosa* L. before inoculation with tobacco mosaic virus resulted in progressively fewer lesions. In contrast, Samuel and Bald (7) present evidence that frequent watering of plants of *N. glutinosa* grown in small pots during the summer months resulted in "hard" plants that had fewer lesions when inoculated with tobacco mosaic virus or with spotted wilt virus than did plants that had been watered less frequently.

Although not reported by these workers, the various differences attributed to environments could have been the result of different osmotic pressures of the fluids of the plant tissues as affected by exposure to the different environments.

MATERIALS AND METHODS.—The materials and methods discussed in this section are common to the majority of experiments. Special methods will be discussed under experimental results.

With the exception of the work on the effects of osmotic pressure on other hosts, the plants used were the Pinto variety of the common bean, *Phaseolus vulgaris* L. Seeds of test plants were sown in sterilized soil in 4-in. clay flower pots. The plants were used 13 days later.

Inoculum consisted of the green-mottling strain of tobacco mosaic virus (TMV) extracted from diseased 4- to 10-week-old plants of *Nicotiana tabacum* L. and

purified by differential centrifugation at 5000 and 20,000 rpm. The virus pellet so obtained was resuspended in phosphate buffer (pH 7). One ml of the purified virus buffer mixture was mixed with 1000 ml of a 0.1 per cent (by weight) K_2HPO_4 solution.

The unifoliate leaves of the bean plants were dusted with No. 600-grit Carborundum and swabbed with a small piece of cheesecloth that had been dipped in the inoculum.

Treatments in all experiments were randomized, or a Latin square or split-block arrangement was used, depending upon the size and nature of the experiments. Analysis of variance was employed for most of the data; however, in tests on association or in 2 treatment comparisons, the regression test and the *t* test, respectively, were used. For isolation of comparisons with a single degree of freedom in the analysis of variance test, an orthogonal comparison was used whenever possible.

EXPERIMENTAL RESULTS.—*Effect of osmotic pressure of different solutions on number of lesions on Pinto beans.*—Osmotic pressures for all electrolytes were calculated from freezing-point depressions of aqueous solutions according to the method of Hall, Miles, and Sherrill (3). Theoretical osmotic pressures were calculated for the sugar solutions; these agreed very closely with those calculated from freezing-point depression data.

1) *Detached-leaf experiments.*—In this laboratory, a detached-leaf method as commonly used in virus inactivator studies (5, 9, 10) gave variable results in tests on virus inhibition due to systemic chemicals. Since the variability appeared to be due to sugar that had been used in the solution, the nature of the action of the sugar was studied further.

Primary leaves were detached from 9-day-old Pinto bean plants, inoculated with TMV, and floated on various sucrose solutions or placed on sand containing the sucrose solutions. Also, the petioles of other leaves were placed in sucrose solutions or in sand containing sucrose solutions. In the check treatments, distilled water was substituted for the sucrose solutions.

Statistically significantly fewer lesions developed on leaves that had received a sucrose treatment without sand than on corresponding controls (Table 1). When sand was incorporated into the solutions, no significant difference was found. Apparently the inhibitory action of the sucrose was suppressed in some way by the sand. It is recommended that sand not be used when a detached leaf method is used for screening chemicals, as the action of the chemicals may in some way be affected by the sand.

2) *"Whole" and "severed" plants.*—To ascertain the inhibitory effects of sucrose when an entire plant was used, roots of bean plants were washed free of soil and the plants (plants with roots) placed in tap water (check), in 1 per cent glucose, in 1 per cent sucrose, or in 2 per cent sucrose. Another group of plants (plants without roots) were severed at the crown and the shoots placed in other flasks containing these same solutions. Plants were inoculated with TMV 2 days later.

TABLE 1.—*Number of lesions on detached Pinto bean leaves inoculated with tobacco mosaic virus and exposed in various ways to distilled water or to a 2 per cent sucrose solution*

Leaf treatment	Number of lesions[a] when substrate was as indicated	
	2% Sucrose	Water (check)
Floated on liquid substrate	5**	66
Placed on sand containing substrate	36	33
Petioles placed in substrate	4*	48
Petioles placed in sand containing substrate	34	53

[a] Asterisk indicates that the difference between the indicated figure and that for the corresponding check is statistically significant at the 5 per cent level; double asterisk indicates statistical significance at the 1 per cent level.

In both groups of plants, fewer lesions (statistically significant at the 5% level) developed on plants placed in 2 per cent sucrose or in 1 per cent glucose than on those placed in distilled water (Table 2). In contrast, the difference between the number of lesions on plants in 1 per cent sucrose and that on the check plants was not statistically significant in either series. Although the same trend occurred in both of the series, a significantly higher mean number of lesions resulted when the severed-root method was used. This method is recommended and was used in other studies of this nature.

Theoretical osmotic pressures for the various solutions were calculated (Table 2). The osmotic pressures of the 2 solutions resulting in fewest lesions were close to 1.3 atmospheres, whereas that of the solution that did not affect lesion number was about 0.6 atmospheres.

To test whether the reduction could be attributed to osmotic pressure, the effects on lesion numbers of solutions of chemicals of known osmotic pressures were determined.

3) *Sucrose.*—Pinto bean plants without roots were placed in distilled water (osmotic pressure approxi-

TABLE 2.—*Mean number of lesions per leaf of Pinto bean plants with and without roots placed in various sugar solutions and inoculated with tobacco mosaic virus*

Treatment	Calculated osmotic pressure (atmospheres)	Mean No. of lesions[a] per leaf	
		Plants without roots	Plants with roots
Tap Water (check)	0	15.9	7.0
Sucrose, 1%	0.65	14.2	3.9
Sucrose, 2%	1.31	5.9*	1.6*
Glucose, 1%	1.24	4.1*	0.6*

[a] Asterisk indicates that the difference between indicated figure and that for the corresponding check is statistically significant at the 1 per cent level.

mately 0.0) and in sucrose solutions with osmotic pressures of from 0.2 to 2.0 atmospheres (increments of 0.2 atmospheres). Two days later, the plants were inoculated with TMV. The mean numbers of lesions per leaf were recorded 4 days later and are presented with a regression line in Figure 1, A.

In general, a direct negative association existed between the mean numbers of lesions and the osmotic pressure, although an upward trend occurred near 2.0 atmospheres.

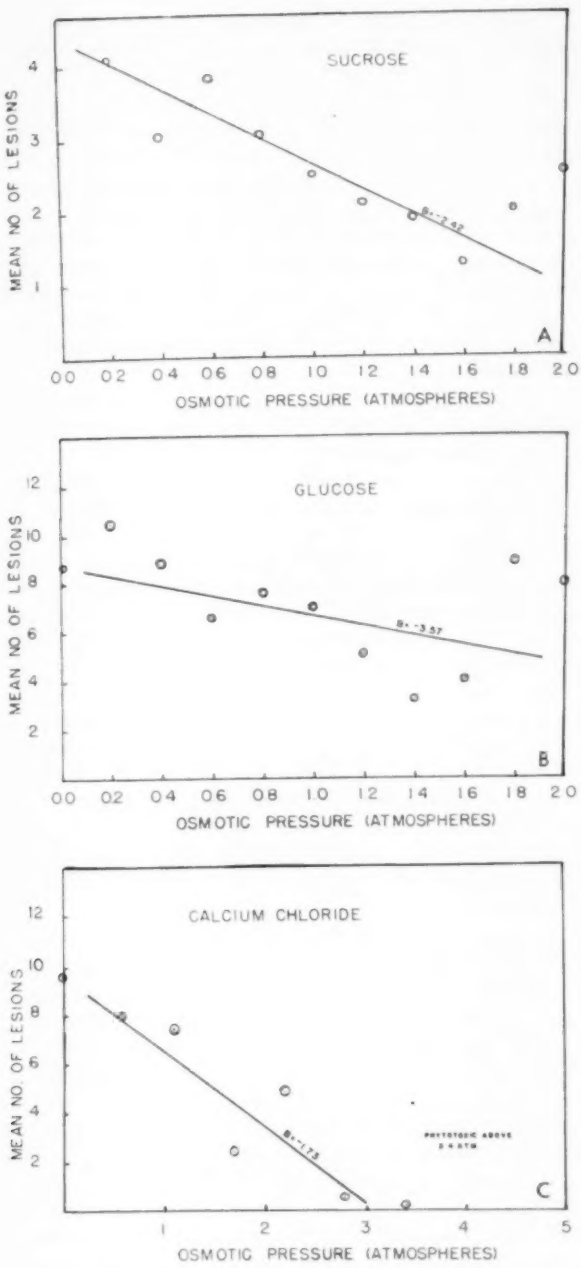

FIG. 1. Mean number of lesions per leaf on Pinto bean plants inoculated with tobacco mosaic virus and placed in solutions of various osmotic pressures of A) sucrose, B) glucose, and C) calcium chloride.

A statistical analysis was made of the data on the differences between number of lesions on check plants and on those treated at 3 lecels of osmotic pressure: those near 2.0 atmospheres, 0.6 atmospheres (that calculated for 1 per cent sucrose), and 1.3 atmospheres (that calculated for 1 per cent glucose or 2 per cent sucrose). Significantly fewer lesions were found at the 1.3 atmosphere region, but not at 0.6 or 2.0 atmospheres, than on plants placed in distilled water.

4) *Glucose.*—The above procedures were used with solutions of glucose. Again, a direct negative association was found between the mean number of lesions and the osmotic pressure (Fig. 1, B), with a rise in the curve at 2.0 atmospheres as was true in the sucrose series.

5) *Fructose and maltose.*—Bean plants were placed in maltose or fructose solutions of 0.6, 1.3, and 2.0 atmospheres and in distilled water. The mean number of lesions per leaf for solutions of 0.0, 0.6, 1.3, and 2.0 atmospheres were 11.25, 3.16, 1.15, and 4.05, respectively, for solutions of fructose and 11.25, 4.8, 3.95, and 7.7, respectively, for solutions of maltose.

Statistically significant differences, when compared with the check, were found at all fructose levels and at 0.6 and 1.3 atmospheres with maltose. The fewest lesions developed at 1.3 atmospheres with both sugars.

A definite effect of osmotic pressure on number of lesions formed was found at 1.3 atmospheres with all sugars tested. An adverse effect on lesion development may occur at pressures greater or less than 1.3 atmospheres, depending on the individual sugar. In general, a direct negative association exists between the number of lesions and osmotic pressure—the number of lesions decreasing as osmotic pressure increases.

6) *Salts.*—a) *$CaCl_2$.*—Procedures similar to the above were used in tests with bean plants placed in $CaCl_2$ solutions of osmotic pressures of 0.6, 1.1, 1.7, 2.2, 2.8, 3.4, 4.5, 6.6, 7.5, 8.8, 9.0, 9.8, and 10.6 atmospheres. Solutions above 3.4 atmospheres were phytotoxic. The results of the remaining plants are given in Figure 1, C.

A direct negative association existed between the mean number of lesions and osmotic pressure. When the regions near 0.6, 1.3, and 2.0 atmospheres were considered, only the lesion counts at 1.3 and 2.0 atmospheres differed significantly (5% level) from those of the checks. Fewest lesions developed at 1.3 atmospheres.

b) *K_2SO_4.*—Bean plants in distilled water (check) and in K_2SO_4 solutions of osmotic pressures of 0.6, 1.3, and 2.0 atmospheres were tested by the procedures described above. The mean number of lesions per leaf for the check and treatments were, respectively, 2.1, 13.9, 11.6, and 32.2. The differences between the number of lesions produced on check plants and that on plants placed in each of the salt solutions were

all statistically significant. Other than on the check, fewest lesions occurred at 1.3 atmospheres. Either the K^+ ion or the SO_4^{++} apparently favors lesion formation and could counteract the inhibitory effects of osmotic pressure. The least favorable effect of K_2SO_4 occurred at the osmotic pressure where inhibition is usually found (1.3 atmospheres).

Other bean varieties.—The effect of osmotic pressure of sucrose solutions was tested with bean plants of varieties other than Pinto; these included White Creaseback, Great Northern, and Michilite. The means of the numbers of lesions for treatments of 0.0, 0.6, and 1.3 atmospheres were, respectively, 4.3, 10.1, and 7.2 for White Creaseback; 2.9, 9.9, and 7.4 for Michilite; and 2.0, 3.7, and 2.1 for Great Northern. When compared with their checks (0.0 atmospheres), a statistically significant increase in lesion number was found at 0.6 atmospheres with all varieties and at 1.3 atmospheres with Michilite. Although the typical minimum at 1.3 atmospheres found with Pinto beans did not occur, osmotic pressure appears to have some effect on lesion development on other bean varieties.

Time of inoculation.—In all previous experiments, the plants were placed in the various solutions for 2 days before being inoculated with TMV. In further tests, Pinto bean plants were inoculated immediately, 1 day and 2 days after they were placed in flasks containing water or solutions of glucose, sucrose, or $CaCl_2$, each with osmotic pressures of 0.6 and 1.3 atmospheres. The mean number of lesions that developed are presented in Table 3.

When plants were placed in the various solutions and inoculated with TMV immediately, significant differences occurred only with the sucrose treatments, where fewer lesions were found at 1.3 atmospheres when compared to the water check. When plants were inoculated 1 day after being placed in the solutions, statistically significant differences occurred only with the glucose and sucrose treatments. When compared with their respective water checks, more lesions occurred at 0.6 atmospheres with glucose and at 1.3 atmospheres with sucrose. If plants were exposed to the solutions and inoculated 2 days later, statistically significantly fewer lesions were found at 1.3 atmospheres with glucose, sucrose, and $CaCl_2$ and at 0.6 atmospheres with $CaCl_2$ as compared with their respective water (0.0 atmospheres) checks.

Table 3.—*Number of lesions per leaf of Pinto bean plants inoculated with tobacco mosaic virus (TMV) at various intervals after being placed in solutions of various osmotic pressures*

Solution	Osmotic pressure (atmospheres)	Mean number of lesions[a] per leaf when plants were inoculated at the indicated interval after being placed in solution		
		0	1 day	2 days
Glucose	0.0	3.9	1.6	2.5
	0.6	4.0	2.8*	1.6
	1.3	4.1	1.4	1.3*
Sucrose	0.0	4.3	1.6	2.0
	0.6	4.2	1.3	1.4
	1.3	2.0**	3.2**	0.4**
$CaCl_2$	0.0	2.5	2.7	3.9
	0.6	2.8	3.4	2.2*
	1.3	2.5	1.5	2.1*

[a] Single asterisk indicates that the difference between the indicated figure and that for the corresponding check (0.0 atmosphere) is statistically significant at the 5 per cent level; double asterisk indicates significance at the 1 per cent level.

Apparently, any effect of osmotic pressure on lesion number is dependent on the period of time the suscept is in the solution, and this period varies with the nature of the solution. In evaluations of systemic virus inhibitors, the time of inoculation should be considered in standardization of procedure.

Effect of osmotic pressure on inoculum.—The effects of osmotic pressure on number of lesions could result from direct effect on the virus or from an indirect effect on the host plant. If osmotic pressure affects the virus directly, fewer lesions should develop on plants inoculated with TMV in a solution with an osmotic pressure of 1.3 atmospheres than when inoculated with TMV in water.

Pinto bean plants were placed in sucrose solutions with osmotic pressures of 0.0, 0.6, 1.3, and 2.0 atmospheres and inoculated with TMV suspended in distilled water. Another group of plants was placed in flasks containing distilled water and inoculated with TMV suspended in sucrose solutions of either 0.6, 1.3, or 2.0 atmospheres. A third group of plants was placed in flasks containing the various sucrose solutions and inoculated with the virus suspended in different sucrose solutions. The usual small number of lesions occurred at 1.3 and at 2.0 atmospheres when the plants were placed in the sucrose solutions and inoculated with the virus-water mixture. When the plants were placed in distilled water and inoculated with the virus-sucrose mixtures, no statistically significant differences occurred among the 3 inocula. When plants were placed in the sucrose solutions and inoculated with the virus-sucrose mixtures, significantly fewer lesions developed on plants in the 0.6 atmosphere solutions than on plants in any other sugar solution.

Apparently the effects of osmotic pressure on lesion number results primarily from an effect on the host.

Cryoscopic determination.—The actual osmotic pressure of expressed sap of leaf tissue of Pinto bean plants that had been placed in solutions of sucrose with osmotic pressures of 0.0, 0.6, and 1.3, were determined by the cryoscopic method (2). Sap was extracted from the leaflets with a Carver plant press after the plants had been in the solutions for 2 days. The osmotic pressures were 7.2, 8.6, and 11.7 atmospheres, respectively, for plants placed in solutions with osmotic pressures of 0.0, 0.6, and 1.3 atmospheres.

Yarwood (13) found that Pinto bean pulvini were resistant to 3 fungi and to 5 viruses (including TMV) but that the blades were susceptible. His results with TMV were duplicated in the present tests; no lesions

occurred on the pulvini, a few on the petioles, and many on the blades. If resistance of the pulvini is correlated with the osmotic pressure of the tissue, the osmotic pressure should be around 11.7 since this was the actual value of the extract of leaflets of bean plants placed in the sucrose solution of 1.3 atmospheres (the osmotic pressure that usually results in the smallest number of lesions). The actual osmotic pressures of the extract of blade, petiole, and of pulvini tissue of Pinto beans growing in soil in the greenhouse were determined cryoscopically. The osmotic pressures in atmospheres were as follows: blades, 7.3; petioles, 8.9; and pulvini, 10.7.

The resistance of bean pulvini to TMV may be a result of the osmotic pressure of the plant fluids in the pulvini.

DISCUSSION.—The colligative properties of solutions may greatly affect lesion number and should not be overlooked in evaluation of various substances for virus disease control. It is evident that in any virus work in which plants are subjected to different chemical solutions, the osmotic pressure of the solutions should be standardized as much as possible. Further study of the exact nature of the effects of osmotic pressure, such as the effect on amino acid content, and on the actual osmotic pressures of the plant fluids when exposed to various osmotic pressure treatments is warranted.

Although the effect of osmotic pressure on lesion development on bean plants varied with the variety, it appeared to have some effect with all bean varieties tested. It is very possible that osmotic pressure has some effect on most plants and that these effects are in some way related to host-pathogen relationships, especially in the case of viruses, and should therefore be thoroughly investigated.

Osmotic pressure could be related to the resistance found in Pinto bean leaf pulvini to various fungi and viruses by Yarwood (13), since it was demonstrated that the osmotic pressure of fluids extracted from the pulvini approximated that of fluids extracted from the leaves of plants placed in a 1.3 atmosphere solution (the osmotic pressure at which the fewest lesions occurred).

The data suggest that of the solutions used osmotic pressure does not affect TMV directly; however, with other chemicals or with other viruses, osmotic pressure might affect a virus directly, such as by denaturation of the virus protein. Osmotic pressure could affect the physiology of the host such that the virus does not increase *in vivi* or such that typical lesions are not formed even though the virus does increase.

Osmotic pressure could be the explanation for the observations of Bawden and Roberts (1) who found a smaller number of lesions on test plants kept in the sun prior to inoculation as opposed to the number on those kept in the shade. They postulated that photosynthetic products of the assay hosts were in some way inhibitory to lesion formation; this could be due to the osmotic effects of this "photosynthetic product." In a like manner, the effect of time of day of inoculation reported by Mathews (4) and Yarwood (14) could be explained on the basis of osmotic pressure, as could the small number of lesions on inoculated detached bean leaves floated on 10 per cent sucrose that was reported by Yarwood (12).

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LITERATURE CITED

1. BAWDEN, F. C., AND F. M. ROBERTS. 1947. The influence of light intensity on the susceptibility of plants to certain viruses. Ann. Appl. Biol. **34**: 286-296.
2. FINDLAY, A. 1929. Practical physical chemistry. Ed. 4. Longmans, Green, and Co. London, New York. 298 p.
3. HALL, R. E., AND M. S. SHERRILL. 1928. Freezing-point lowerings of aqueous solutions. In International critical tables of numerical data. Physics, Chemistry and Technology. V. 4. National Research Council, Washington. p. 254-264.
4. MATHEWS, R.E.F. 1953. Factors affecting the production of local lesions by plant viruses. I. The effect of time of day. Ann. Appl. Biol. **40**: 377-383.
5. NICHOLS, C. W. 1954. Absorption of thiouracil by tobacco leaf tissue and inhibition of tobacco mosaic virus multiplication. Phytopathology **44**: 92-93.
6. SAMUEL, G. 1931. Some experiments on inoculating methods with plant viruses and on local lesions. Ann. Appl. Biol. **20**: 70-99.
7. SAMUEL, G., AND J. G. BALD. 1935. Further studies on quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70-99.
8. SAMUEL, G., R. J. BEST, AND J. G. BALD. 1935. Further studies on quantitative methods with two plant viruses. Ann. Appl. Biol. **22**: 508-524.
9. SCHLEGEL, D. E., AND T. E. RAWLINS. 1954. Inhibition of tobacco mosaic virus by an antibiotic from an actinomycete Nocardia species. Phytopathology **44**: 328-329.
10. SCHNEIDER, I. R. 1954. The effect of purines, purine analogs and related compounds on the multiplication of tobacco mosaic virus. Phytopathology **44**: 243-247.
11. TINSLEY, T. W. 1953. The effects of varying water supply of plants on their susceptibility to infection with viruses. Ann. Appl. Biol. **40**: 750-760.
12. YARWOOD, C. E. 1952. Some relations of carbohydrate level of the host to plant virus infection. Am. J. Botany **39**: 119-124.
13. YARWOOD, C. E. 1954. Resistance of bean leaf pulvini to fungi and viruses. Phytopathology **44**: 64.
14. YARWOOD, C. E. 1956. Further aids in virus inoculations. (Abs.) Phytopathology **46**: 32.

FUSARIC ACID AS A WILT TOXIN[1]

Ernst Gäumann

SUMMARY

Fusaric acid, a pyridine-carboxylic acid, is formed not only by specific wilt pathogens such as *Fusarium lycopersici* Sacc. and *F. vasinfectum* Atk. but also by no fewer than 6 members of the family Hypocreaceae. Parasitologically, these belong to very different groups of pathogens. At least two of them also form dehydrofusaric acid; in *in vitro* culture, the saturated fusaric acid appears earlier than the unsaturated dehydrofusaric acid.

Since none of these fungi produces only 1 toxin, the fusaric acid in the several pathogens must function together with other kinds of toxins and with other metabolic products produced by the pathogen; e.g. that produced by *F. lycopersici* acts together with at least 5 and that produced by *Gibberella fujikuroi* (Saw.) Wr. with at least 3.

Fusaric acid production inside the host has, so far, been demonstrated for *F. lycopersici* in tomato plants and for *F. vasinfectum* in cotton plants; in the latter, it has been found in amounts up to 17 mg/kg fresh weight.

The problem of whether the pathogens of wilt diseases, in addition to their enzymatic action, also injure their host plants by means of more or less specific toxins is not yet settled. During the later years of World War II, however, the first specific wilt toxin lycomarasmin, was isolated from *F. lycopersici* Sacc. and defined chemically as a dipeptide (11).

In the English literature there are some errors concerning this toxin. It is stated that it is an ageing product of *F. lycopersici*, appearing in the culture filtrate only after a lapse of 2 months. This idea is due to a misunderstanding of the German text. We normally harvest the lycomarasmin in our cultures at the end of 6–8 weeks, and this has been interpreted to mean that the lycomarasmin is produced only after 6–8 weeks. In fact, lycomarasmin can be demonstrated in the mycelium of *F. lycopersici* as early as the seventh day; in culture filtrates the lycomarasmin content at this time amounts to about 10 mg/l, and it increases to about 200–300 mg/l by the 44th day, when we collect it.

Soon after the isolation of lycomarasmin, it became apparent that the young culture filtrates of *F. lycopersici* must contain at least 1 other toxin in addition to the lycomarasmin, since these culture filtrates inhibit the growth of such bacteria as *Bacillus mesentericus* Trev., *Micrococcus pyogenes* var. *aureus* (Ros.) Zopf, and *Pseudomonas aeruginosa* (Schroet.) Mig., which lycomarasmin alone cannot do. For this reason, we have never stated that lycomarasmin is the wilt toxin, but only that it is a wilt toxin of *F. lycopersici*.

It was necessary, therefore, to find a way of differentiating this second toxin (which, as appeared later, is fusaric acid) from lycomarasmin. The previously mentioned bacteria were not suitable as test organisms, because their reaction is not distinct enough. Moreover, young tomato shoots could not be used in wilt experiments, since both toxins evoke similar effects in tomato leaves.

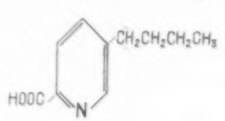
Our work on differentiation of the 2 toxins could not really begin until 7 years later, when a test object that reacts to fusaric acid but not to lycomarasmin was discovered. This differentiating technique, which is based on germination tests with chlamydospores of *Ustilago zeae* (Beckm.) Ung., was developed by Kern and described precisely by Kobel (51) and by Kern (48). Fusaric acid inhibits relatively severely the germination of the chlamydospores of *U. zeae* (the LD_{50} is in the region of 10^{-4} M), whereas lycomarasmin itself is innocuous to such spores.

Thus, when a culture filtrate contains both lycomarasmin and fusaric acid, its content of fusaric acid can be determined *ceteris paribus* by means of its "Ustilago-activity," i.e. its inhibitory effect on the germination of the chlamydospores of *U. zeae*. By this means, fusaric acid was subsequently recognized as an important pathogenic agent in several instigators of wilt diseases (28).

The present paper discusses the chemistry and mode of formation of fusaric acid, its formative and operative range, and certain disease symptoms evoked by it. In a later paper we shall try to describe the ways in which fusaric acid produces its effects.

THE CHEMISTRY OF FUSARIC ACID.—Fusaric acid was first isolated by Yabuta, Kambe, and Hayashi (82) as a metabolic product of an insignificant fungus, *Fusarium heterosporum* Nees, that is found all over the world as a saprophyte or nonspecific parasite on grasses, apples, ergots, etc. Eighteen years later it was recognised by Gäumann, Naef-Roth, and Kobel (28) as an important wilt toxin of *Fusarium lycopersici*, *F. vasinfectum* Atk., and *Gibberella fujikuroi* (Saw.) Wr.

Chemically it is a pyridine-carboxylic acid, namely 5-butylpicolinic acid, with the empirical formula $C_{10}H_{13}O_2N$ and a molecular weight of 179 (82):



[1] Accepted for publication January 23, 1957.
The author is indebted to the Swiss National Foundation for Scientific Research for a grant.

Up to the present this constitutional formula, which made possible the synthesis of the fusaric acid (39,62,63), has remained undisputed.

Certain differences occurred only in relation to the melting point of the compound. Pure fusaric acid has a melting point of 98°–100°C (39,77), but biogenic preparations often contain traces of dehydrofusaric acid and possess therefore a somewhat higher melting point (up to 109°) (62,63,75,82).

The existence of dehydrofusaric acid in preparations of fusaric acid was demonstrated by Stoll (75). In the case of certain fungi that produce fusaric acid, e.g. *G. fujikuroi*, culture filtrates cause in the interveinel areas in the leaves of young tomato shoots a chlorosis that never appears as such in wilt experiments with pure fusaric acid. Researches on extraction and purification of these culture filtrates led to the preparation of pure dehydrofusaric acid (5-butylenicpicolinic acid) with the empirical formula $C_{10}H_{11}O_2N$, a molecular weight of 177, and a melting point of 118–120°. Hydrogenation of dehydrofusaric acid with platinum and glacial acetic acid converts it to fusaric acid (76).

Surprisingly, the unsaturated dehydrofusaric acid appears after the appearance of saturated fusaric acid in culture filtrates of *F. lycopersici* (77) as well as in those of *G. fujikuroi* (76). It is not yet known if dehydrofusaric acid is produced *de novo* independently of the synthesis of fusaric acid, or if it remains in the culture filtrates as a stage of an incomplete fusaric acid synthesis.

For the technical production of fusaric acid, culture filtrates of the fungi are brought to pH 4.0 and thoroughly extracted with ethyl acetate. The ethyl acetate extract is evaporated and the residue dissolved in a little water and made alkaline with bicarbonate (of about pH 8–8.5). To remove impurities, the alkaline water solution is shaken twice with half its volume of ether, again acidified, and thoroughly extracted with ether in a fluid-extration apparatus to obtain the acids. After evaporation of the ether these acids are readily crystallized. Usually, a mixture of fusaric acid and dehydrofusaric acid is obtained, but this can be resolved by Craig-distribution between water and ethyl acetate (70,76).

A paper chromatographic method for determining small amounts of fusaric acid was devised by Zähner (86,87).

THE NUTRITIONAL PHYSIOLOGY OF FUSARIC ACID PRODUCERS.—Study of the metabolism of the wilt disease pathogens in which we are interested dates back to the researchers, amongst others, of Sherwood (73), White (80), Ahmet (1), and Luz (59). More recently, such work has been reported by Gottlieb (36), Stoll (75), Flück and Richle (22), and Sanwal (71). Detailed comparisons of the results of these investigations cannot be made, because the experiments were carried out at different times, with different strains, and under different conditions.

The metabolism of fusaric acid producers.—Since

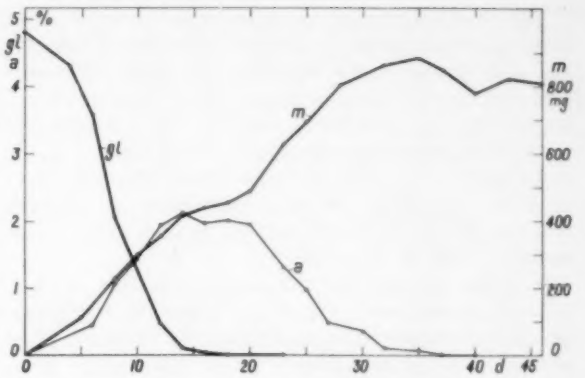


FIG. 1. Mycelial growth, sugar consumption, and alcohol formation of *Fusarium lycopersici* in a modified Richard's solution. *m*) Mycelial dry weight in mg. *gl*) Glucose content of culture filtrate as percentage of nutrient solution. *a*) Ethyl alcohol content as percentage of nutrient solution. *Abscissa:* days after seeding of cultures (After Luz (59)).

the fusaric acid molecule contains only carbon, nitrogen, oxygen, and hydrogen, the carbon and nitrogen metabolism of the pathogens concerned must play a decisive role in the biosynthesis of these compounds.

1) Sources of carbon.—The glucose provided *in vitro* is depleted by *F. lycopersici* in some cases in about 18 days (Fig. 1, curve *gl*) and in other cases (71) in about 36 days, whereas it is depleted by *G. fujikuroi* within 11 days (75).

Although no direct relation can be demonstrated between carbohydrate metabolism and fusaric acid synthesis, the production of fusaric acid is conditioned by the ratio of carbon and nitrogen available in the nutrient solution; thus, in Richard's nutrient solution, *F. lycopersici* produces more fusaric acid in media with 2.5 per cent C and 0.5 per cent N than in media containing 2.5 per cent C and 1 per cent N or 5 per cent C and 0.5 per cent N (71). The optimal C:N ration seems to be about 5:1.

In stock cultures in Erlenmeyer flasks where aeration of the solution is relatively poor, *F. lycopersici* converts some of the glucose provided into ethyl alcohol (Fig. 1, curve *a*), which in turn serves as a carbon source and disappears in about 37 days (59).

It is probable that ethyl alcohol is formed not only *in vitro* but also under the nutritional conditions inside tomato shoots, where it may alter considerably the disease-disposition of these shoots. Thus, if shoots of the resistant tomato variety Jefferson are placed in conidial suspensions of *F. lycopersici* containing 1.0, 0.5, or 0.2 per cent ethyl alcohol, they become characteristically wilted in 12–15 days, whereas the controls, and the parallels treated with similar concentrations of methyl, isopropyl, or *n*-butyl alcohol, remain unaffected (72). Thus, ethyl alcohol has a specific toxic effect, and a concentration of only 0.2 per cent suffices to alter the reaction type of tomato shoots from that characteristic of resistant varieties to that of susceptible ones.

The threshold value at which this toxic effect of ethyl alcohol operates is probably much lower than 0.2 per cent, perhaps only one-tenth as much. It seems probable that *F. lycopersici* can effect such a concentration *in vivo*, inside the vessels. For example, the greatest *in vitro* concentration of ethyl alcohol in the experiment illustrated in Figure 1 was 2.1 per cent; if only 1 per cent of this production is achieved *in vivo*, the threshold of activity in the host tissues probably will be exceeded.

It is not improbable, therefore, that ethyl alcohol, as well as ethylene (20), plays a part in the pathogenesis of tomato wilt; in contrast to ethylene, ethyl alcohol does not act directly as an independent toxin on the host tissues but only as a companion substance that conditions the host plant for the action of other pathogenic substances.

F. lycopersici can also utilize the carbon from certain amino acids, such as aspartic acid, glutamic acid, and glycine; these can serve as the only source of carbon (36,71). If certain amino acids are the only source of both carbon and nitrogen, the fungus grows without developing its full metabolism; e.g. it forms considerable amounts of fusaric acid if it is supplied with glycine but not when aspartic acid or glutamic acid is its sole source of carbon and nitrogen (71).

2) Sources of nitrogen.—Inside the host, both inorganic and organic sources of nitrogen are available to wilt pathogens.

If either *F. lycopersici* (59, 71) or *G. fujikuroi* (75) is cultivated *in vitro* in a solution of appropriate pH containing ammonium nitrate, it utilizes mainly the nitrate-N in the first period of growth lasting some 2–3 weeks (Fig. 2); later, it also utilizes the ammonia-N. A direct relation between nitrate utilization and fusaric acid production cannot, however, as yet be established (Fig. 2).

This preference for nitrate nitrogen may explain an observation by Clayton (12) that the differential

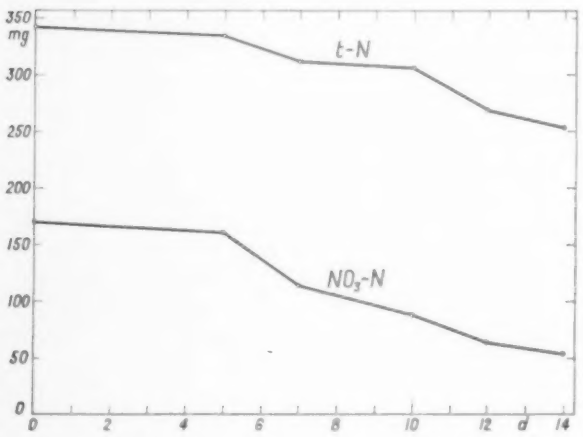


FIG. 2. The course of nitrogen utilization by *Fusarium lycopersici* in a culture solution containing nitrogen in the form of ammonium nitrate. *Abscissa:* time in days. *Ordinate:* total nitrogen (t-N) and nitrate nitrogen (NO_3-N) in culture fluid (After Luz (51)).

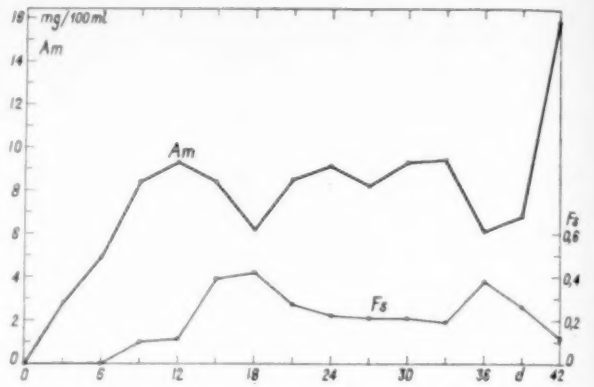


FIG. 3. The content of amino nitrogen (curve *Am*) and of fusaric acid nitrogen (curve *Fs*) in the culture filtrate of *Fusarium lycopersici* grown in a modified Richard's nutrient solution containing ammonium nitrate. *Abscissa:* time in days. *Ordinate:* N content in mg/100 ml of culture filtrate. The curve for fusaric acid N was increased by a factor of 10. (After Sanwal (71)).

incidence of disease among tomato plants grown under varying conditions of soil moisture was accompanied by differences in the nitrate content of their tissues; with a soil moisture of about 24 per cent, when all the test plants became diseased, the basal parts of the shoots were rich in nitrate nitrogen, whereas plants grown at a soil moisture of about 14 per cent, when no disease occurred, contained only traces of nitrate nitrogen. In any case, it would be worthwhile to re-examine these observations by Clayton.

Most of the work on organic sources of nitrogen has been carried out on amino acids. In a culture of *F. lycopersici* on Richard's nutrient solution containing N in the form of ammonium nitrate (Fig. 3), the content of a amino-N and that of fusaric acid in the culture fluid varied in opposite directions (71). The amino-N content of the culture filtrate reached a first maximum in 12 days. Subsequently, the fusaric acid content in the culture filtrate increased rapidly and the amino-N content decreased *pari passu*. For reasons as yet obscure, the content of fusaric acid then declined, whilst that of amino-N increased. Between the 33rd and 36th day, this process was repeated: the amino-N content decreased and the fusaric acid content increased. After the 36th day, the amino-N content became quite high, probably because of extensive autolysis. These relations, however, are not yet clear (22); it seems that the fluctuations in the amounts of fusaric acid produced run counter to those in the measurable amounts of citrulline and alanine in the culture filtrates (Table 1).

3) Consecutive changes in mycelial weight.—In cultures of *F. lycopersici* and of *G. fujikuroi*, the mycelial weight steadily increases during the first 3–5 weeks (Fig. 1, curve *m*); therefore, the weight is not directly influenced by variations in the carbon and nitrogen supply as discussed above. After this period, however, autolysis predominates over growth;

TABLE 1.—*The amino acid content of the culture filtrate and of the aqueous mycelial extract of Fusarium lycopersici grown in nutrient solution containing ammonium nitrate as the only source of nitrogen (After Flück and Richle (22))*

Amino acid	Relative amounts* of amino acids when cultures were of indicated age (days)							
	In culture filtrate				In aqueous mycelial extract			
	7	18	24	34	7	18	24	34
Asparagine	1	1	1	1	1	1	1	1
Aspartic acid	3	3	3	3	3	3	3	3
Glutamic acid	3	3	3	3	3	3	3	3
Serine	1	1	1	1	1	1	1	1
Glycine	1	1	1	1	2	2	2	2
Alanine	1	2	3	1	3	2	3	2
Citrulline	0	1	2	0	2	1	2	0
Threonine	0	0	0	0	2	2	2	2
Valine	0	0	0	0	1	1	1	1
Methionine	0	0	0	0	1	1	1	1
Leucine	0	0	0	0	1	1	1	1
Proline	0	0	0	0	1	1	1	1
Phenylalanine	0	0	0	0	0	0	0	0

*0 = not detectable, 1 = trace, 2 = appreciable amounts, and 3 = abundant.

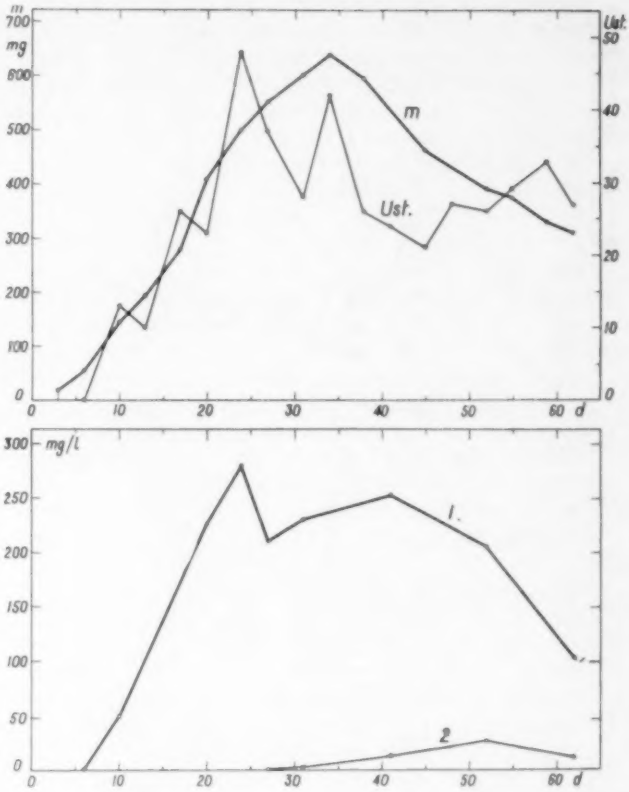


FIG. 4. The time relations of mycelial growth and of toxin formation of *Fusarium lycopersici* in a modified Richard's nutrient solution. *Abscissa:* period of culture in days. *Ordinates:* curve *m*) average mycelial dry weight in mg per flask; curve *Ust.*) Ustilago-activity of the culture filtrate; curve *1*) content in the culture filtrate of pure crystallized fusaric acid; curve *2*) content in the culture filtrate of pure crystallized dehydrofusaric acid in mg/l. (After Stoll et al (77)).

e.g. in Figure 1 the loss of mycelial weight amounts only to about 8 per cent, in Figure 4 to about 23 per cent, whereas in other cultures of *F. lycopersici* it may, in the same length of time, amount to about 40 per cent (71). *G. fujikuroi* (Fig. 5) shows similar fluctuations (75).

This dominance of autolysis over increase shows itself also in the content of the ash constituents of the mycelium; in *F. lycopersici*, it amounts initially to about 8 per cent of the dry weight, then falls gradually to 7–7.5 per cent, and after 14 days drops suddenly (within 48 hours) to about 1.8 per cent (Fig. 6). Potash and phosphoric acid are primarily concerned in this diminution, since the potash content of the mycelium drops from 2.6 to 0.5 per cent within the critical 2 days, and the phosphoric acid content drops from 4.0 to 1.2 per cent (59).

Doubtless, autolysis of single cells starts in all fungal cultures immediately after the onset of growth, but in *F. lycopersici* the breaking down of hyphae by about the 14th day must suddenly take on such catastrophic proportions that macrochemically detectable amounts of important ash constituents return to the nutrient solution from inside the cells. Plasmolytic experiments show that many fungal cells are shrunken and no longer plasmolysable after only 8–10 days in culture; after 2 weeks in culture, this cell mortality becomes so prevalent that it can be detected macroscopically by the appearance of the colony (59).

In *G. fujikuroi*, no detailed study has yet been made of these events, but it would seem probable that they follow a similar course; thus the nitrate content of a culture filtrate increases again after about a 3-week culture period at exactly the same time that the mycelial weight begins to decrease because of the predominance of autolysis (Fig. 7). These nitrates must either have been stored as such in the fungal cells or have been formed anew as end stages in the autolysis of nitrogenous compounds (75).

The time-relations of fusaric acid production in vitro.—The ways in which the several producers of fusaric acid achieve its biosynthesis are still obscure. Thus, we do not yet know whether the stages of formation that are used as steps in the laboratory synthesis of fusaric acid are actually traversed by the pathogens. It is, however, quite clear that fusaric acid is not a product of autolysis, since the data in Figures 4 and 5 indicate no direct connection between mycelial dry weight and the fusaric acid content of the culture solution.

It is remarkable that fusaric acid and dehydrofusaric acid, in contrast to lycomarasmin, have never been demonstrated inside the fungal hyphae. Admittedly, current methods of disintegrating mycelium afford no guarantee that the material of the cell will be obtained in natural form; nevertheless, it appears that traces of both these toxins should have been found

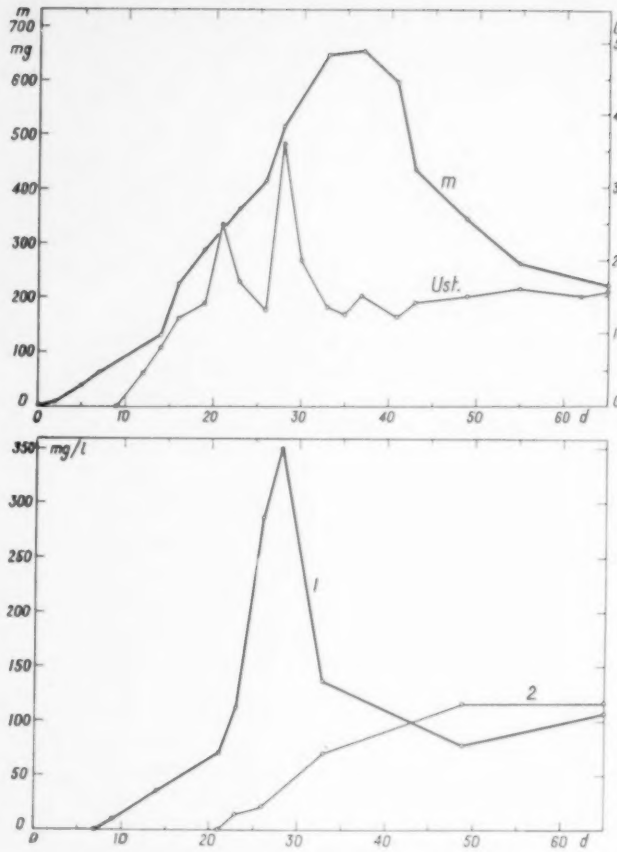


FIG. 5. The time-relations of mycelial growth and of toxin formation of *Gibberella fujikuroi* in a modified Richard's nutrient solution. *Abscissa:* period of culture in days. *Ordinates:* curve *m*) average mycelial dry weight in mg per flask; curve *Ust*) Ustilago-activity of the culture filtrate; curve *1*) content in the culture filtrate of pure crystallized fusaric acid; curve *2*) content in the culture filtrate of pure crystallized dehydrofusaric acid in mg/l. (After Stoll and Renz (76)).

in mycelial preparations.

These gaps in our knowledge about the biosynthesis of fusaric acid are regrettable because the fusaric acid contents of the nutrient solution of cultures of *F. lycopersici* and of *G. fujikuroi*, as indicated by Ustilago-activity, show certain oscillations that are difficult to understand without such knowledge. For instance, in both fungi the Ustilago-activity of culture filtrates reaches a first peak even during the wholly vegetative growth of the pathogen (Fig. 4, 5); then for a time it diminishes, only to reach a second peak some 10 days later (before or during the period at which the mycelial weight is at its maximum); thereafter, it definitely decreases. In very old cultures, probably because of toxic products from mycelial decomposition, the Ustilago-activity sometimes increases again by a small amount (Fig. 4). In exceptional cases, as in an experiment of Sanwal (71) with strain R-5-6 of *F. lycopersici*, the curve of activity may show only a single peak.

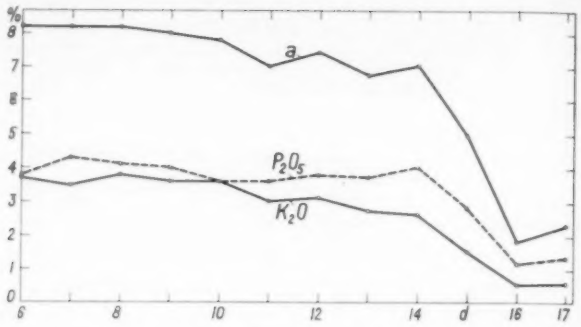


FIG. 6. The content of total ash (*a*), potash (K_2O), and phosphoric acid (P_2O_5) in mycelium of *Fusarium lycopersici*. Each is expressed as percentage of the mycelial dry weight. *Abscissa:* days from seeding of cultures. (After Luz (59)).

If the pathogenic activities of *F. lycopersici* and *G. fujikuroi* were also to follow a double-peak rhythm during their parasitic phase within the host, it might be surmised that the first peak would occur at the start of the acute stage of infection and the second peak at the end of the chronic stage of infection. It may be that the sudden break-down that terminates pathogenesis in many wilt diseases is associated with a second activity maximum of the pathogen.

The 2 peaks of the activity curve of some pathogens result from a quantitatively varying production of the 1 toxin during particular periods of growth (a question of redoxpotentials ?); thus, according to unpublished observations of D. Kluepfel and M. Juillard, the gliotoxin content of culture filtrates of *Aspergillus fumigatus* Fres. and of *Trichoderma viride* Pers. shows 2 marked quantitative maxima in both stationary and shaken cultures (Fig. 8).

With other pathogens, however, both quantitative and qualitative changes in toxin activity occur together during the growth of the cultures; thus, during a first phase of development, culture filtrates of *Fusarium culmorum* (W. G. Sm.) Sacc. contain several toxins

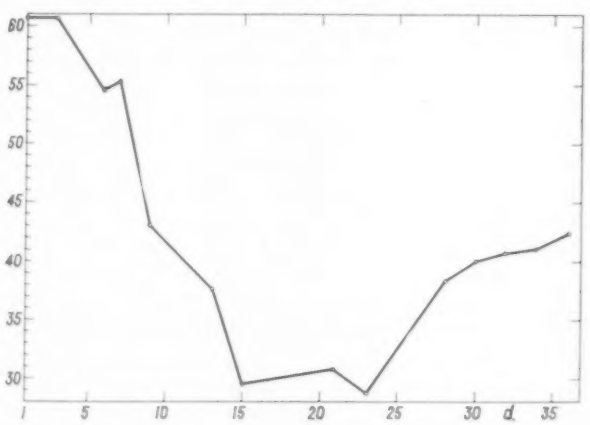


FIG. 7. Consecutive changes in the nitrate content of the culture fluid of *Gibberella fujikuroi*. *Abscissa:* age of culture in days. *Ordinate:* relative nitrate content of culture fluid. (After Stoll (75)).

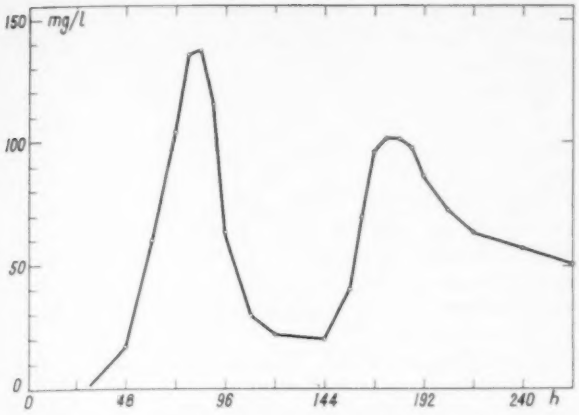


FIG. 8. Quantitative variation of the gliotoxin content in culture filtrates of *Aspergillus fumigatus* Fres. mut. *Helvola* during the growth of the fungal culture. *Abscissa:* culture period in hours. *Ordinate:* gliotoxin content in mg/l. (Original, D. Kluepfel).

that inhibit the growth of *Bacillus subtilis* Cohn and damage the leaves of *Melilotus albus* Desr. and of *Lycopersicon esculentum* Mill. (Fig. 9), whereas only the substance that injures tomato leaves is present in more mature filtrates where it reaches a second maximum of activity (53).

F. lycopersici (77) and *G. fujikuroi* (76) belong to a third group (Fig. 4, 5). The temporary decline in activity between the first and second activity maxima, in particular, finds no explanation in the actual fusaric acid contents; perhaps it is conditioned by secondary products of metabolism that are released by the pathogen into the nutrient solution in that specific phase of growth and that deactivate or bind fusaric acid. Perhaps this change in the pathogen's metabolism is associated with the fact that it is during this phase of development that dehydrofusaric acid first appears in the culture filtrates.

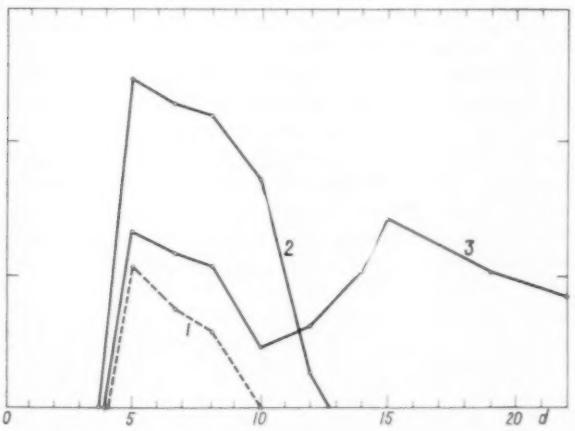


FIG. 9. Toxin content of culture filtrates of *Fusarium culmorum* during the course of its growth. *Abscissa:* culture period in days. *Ordinates:* relative inhibition of *Bacillus subtilis* (curve *1*), injury to leaves of *Melilotus albus* (curve *2*) and of *Lycopersicon esculentum* (curve *3*). (After Landolt (53)).

Special nutritional requirements for the formation of fusaric acid.—Most pathogens are able to form their specific toxins only under particular conditions. In certain cases, toxin synthesis depends on the presence of particular trace elements that are not themselves built into the toxin molecule but into the enzyme systems that are decisive in the formation of the substance in question; e.g. diphtheria toxin is formed only in the presence of iron.

In other cases, the general physiological status of the pathogen determines its formation of toxin and hence its pathogenicity; e.g. certain strains of *Corynebacterium diphtheriae* (Flügge) L. et N. normally cannot produce diphtheria toxin and therefore are non-pathogenic. But if these strains are attacked by Phage B, their metabolism is changed in such a way that they then synthesize diphtheria toxin freely and thereby regain their toxicity and pathogenicity (23, 37). In a similar way, the synthesis of fusaric acid is conditioned by the presence or absence of certain trace substances and by the general physiological status of the pathogen.

Substances favouring fusaric acid production.—The presence of zinc within a very definite and narrow range exerts a favourable influence on fusaric acid production by *F. vasinfectum* (Fig. 10). In the absence of zinc or in its presence below a threshold of 0.08 mg/l, the fungus does not produce fusaric acid. At 0.24 mg/l, the fusaric acid in the culture fluid reaches a maximum. With more than 0.4 mg/l of zinc in the nutritive solution, fusaric acid synthesis is inhibited. Here too, no relation exists between growth of the pathogen and its production of toxin (46).

Similar relationships between the zinc content of the nutrient solution and toxin synthesis exist among the organisms producing medically interesting anti-

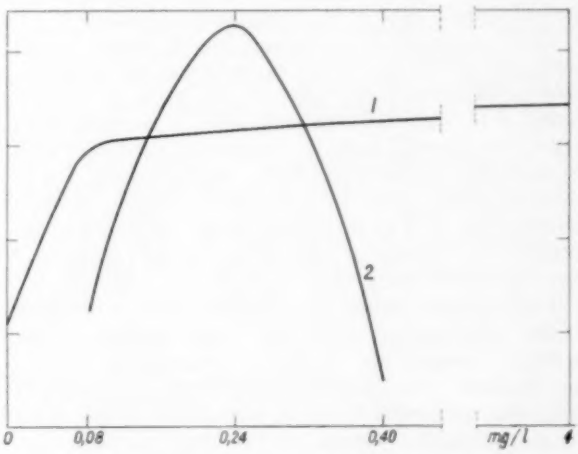


FIG. 10. The influence of the zinc content of the nutritive solution on fusaric acid production by *Fusarium vasinfectum*. *Abscissa:* mg zinc per 1 of nutritive solution. *Ordinates:* mycelial dry weight (curve *1*) and antibiotic activity of the culture filtrates (curve *2*), both in arbitrary units. The antibiotic activity is due mainly to fusaric acid. (After Kalyanasundaram and Saraswathi-Devi (46)).

biotics. *Penicillium notatum* Westl. forms penicillin only in the presence of zinc; in its absence it forms poisonous notatin. *Streptomyces griseus* (Kr.) W. et H. forms streptomycin only in the presence of zinc, iron, and copper (10).

Again, traces of other substances favour fusaric acid synthesis, although the active ingredients are not yet known. "Wad extract," a water extract of crude cotton wool, added to stock cultures of *G. fujikuroi*, accelerates and increases the production of fusaric acid (75). Wad extract also exerts a similar stimulating effect on penicillin production by *Penicillium notatum* (21).

Substances inhibiting fusaric acid production.—The only example so far studied of an inhibitory substance that possibly might be active *in vivo* is tomatin; unfortunately the results of this investigation were negative.

Irving, Fontaine, and Doolittle (44, 45) and Irving (43) isolated from tomato plants a glycoside, tomatin, that has a wide range of antibiotic activity and inhibits the germination of the conidia of the potato pathogen *Fusarium solani* (Mart.) App. et Wr. at a concentration of only 2×10^{-5} M. Since it occurs in the vascular sap of tomato plants at a concentration of about 10^{-4} M (48), it probably constitutes in tomato plants a resistance factor against *F. solani*.

But the specific wilt pathogen of tomato plants, *F. lycopersici*, reacts only slightly to tomatin. The LD_{50} for the germination of its conidia is about 2×10^{-3} M, i.e. 100 times higher than that for *F. solani*. With *F. lycopersici*, neither toxin production *in vitro* nor the activity *in vivo* of lycomarasmin and fusaric acid are reduced by tomatin. This is true for solanin as well as for tomatin (48).

The tomatin content of tomato varieties or of tomato tissues does not, therefore, determine *in vivo* production of fusaric acid by *F. lycopersici*.

The importance of the physiological status of the pathogen for its fusaric acid production.—The fusaric acid production of the different pathogens is also influenced by their physiological status. In such cases vegetative growth may be inversely correlated with fusaric acid production. Thus, under certain conditions of aeration of the cultures, the highly virulent strain R-5-6 of *F. lycopersici* forms much more mycelium than does the weakly pathogenic strain 257 but only about one-fifth as much fusaric acid (48).

The classical example of such a contrary influence of the physiological status of a plant parasite on its mycelial growth and on its production of certain metabolites is found in a distant relative of the wilt pathogen under discussion here, namely in the ergot fungus, *Claviceps purpurea* (Fr.) Tul. *In vitro*, this fungus produces an abundance of mycelium, but there is no appreciable synthesis of alkaloids except for the simpler ones formed by the addition of a N-free radical to lysergic acid (e.g. agroclavin and elymoclavin). In contrast, during its parasitic phase inside ovaries of Gramineae, where its vegetative growth is inhibited, this fungus produces therapeutically valuable peptide alkaloids, like ergotamine and ergotoxin, in amounts up to about 0.2 per cent of its dry weight.

Factors favouring fusaric acid production within the host plant.—No doubt factors similar to those we have discussed for *in vitro* culture will also determine within the host whether or not the pathogen succeeds in forming fusaric acid (and other toxins), thereby attaining its full pathogenicity. If this is true, then all tomato varieties in which *F. lycopersici* can form its specific toxins would, *ceteris paribus*, be susceptible to wilt whereas all varieties in which it could not do so would be resistant.

As an example of what happens in the case of non-congenial pathogens, Scheffer and Walker (72) allowed conidial suspensions of *Fusarium conglutinans* Wr. (the pathogen of wilt, or yellows, of cabbage) to ascend inside shoots of Bonny Best, a tomato variety highly susceptible to tomato wilt. Even after 35 days, the fungus was still present unaltered in the basal portions of the shoots but had induced no disease. In our view, disease was absent from the shoots because they did not enable *F. conglutinans* to form its specific toxins. When it does form these toxins (for example, if one puts tomato shoots in appropriate culture filtrates of *F. conglutinans*), the shoots prove to be highly susceptible. According to our conception, therefore, it is not a toxin resistance that protects the shoots from *F. conglutinans* in the experiments described but merely the circumstance that the shoots do not provide the conditions for the production of toxin by *F. conglutinans*.

Probably the conditions within tomato shoots are similar for different virulent strains of *F. lycopersici*. Certainly the capacity for "generalization" (i.e. for spreading within the host) is very diverse in these, being very high in strain R-5-6 of the U. S. Department of Agriculture and relatively low in strain 257; hence, strain 257 sometimes can be isolated from parts of tomato plants that show no symptoms. It follows, therefore, that the pathogen had achieved colonization but had not produced toxins.

The different wilt-susceptible tomato varieties react alike to lycomarasmin (30) and to fusaric acid (48). Therefore, if these toxins were formed when such varieties are infected by *F. lycopersici*, all would respond identically. The differential response of the different varieties to the pathogen must, therefore, depend upon other resistance factors. In our view, certain wilt-resistant tomato varieties do not provide conditions under which the pathogen can form adequate toxin. Thus, Scheffer and Walker (72) allowed conidial suspensions of a highly virulent strain of *F. lycopersici* to ascend inside shoots of Jefferson, a wilt-resistant tomato variety; the shoots remained infected at the base but did not become diseased. Our interpretation is that this variety did not provide the conditions for toxin synthesis. When the respiration of the shoots is reduced by allowing them to take up 2,4-dinitrophenol or other respiration-inhibiting poisons, they become diseased in the typical manner. Gothoskar et al (34)

assume that whilst respiration continues a substance toxic to the fungus is formed and hinders the advance of the fungus. They suggest that this toxin is so unstable that it cannot be detected in expressed sap and extracts. It may be questioned whether these results cannot be more easily explained on the basis of toxin theory: so long as the variety Jefferson retains its full vitality, its defense reactions prevent the pathogen from forming toxin; as soon as the host is definitely weakened, toxin is produced and the pathogen achieves its full development.

On the same basis, we could explain the results of the grafting experiments of Heinze and Andrus (42). If scions of the highly wilt-resistant tomato variety Pan America are grafted on to stocks of the wilt-susceptible variety Bonny Best, they become as severely diseased as do Bonny Best scions on Bonny Best stocks. But Bonny Best scions on Pan America stocks become diseased only to the same slight degree as do Pan America scions on Pan America stocks or as do self-rooted Pan America plants. The toxin sensitivity of both tomato varieties is equally high, and in our opinion they differ only in their capacity to allow the pathogen to spread and, in particular, to form toxin. On Bonny Best, the pathogen forms toxin and is therefore able to injure the grafted Pan America scion; on Pan America, it achieves neither generalization nor toxin formation and therefore can neither penetrate the Bonny Best scion nor injure it indirectly by means of its toxins. In our opinion, a like situation occurs in the intergeneric grafts of Davis (17).

In our view, it is because the toxin synthesis *in vivo* is controlled by the host that in many cases there exists no direct relationship between the pathogenicity of a pathogen *in vivo* and its toxigenicity *in vitro*: strains highly virulent *in vivo* may be weakly toxigenic *in vitro*, and conversely (30, 48) the extent of pathogenicity *in vivo* depends on how adequately the parasite can maintain its own metabolism inside the host and thus achieve toxin synthesis. Once this is achieved the resistance of the host is soon overcome.

Perhaps with the help of isotope techniques, like that used by Kern and Sanwal (50) and Kern and Kluepfel (49), it will become possible to answer these questions by experimental evidence.

THE RANGE OF FORMATION AND OF ACTIVITY OF FUSARIC ACID.—*The range of formation of fusaric acid.*—Fusaric acid and dehydrofusaric acid, the members of the fusaric acid group to be discussed here, have been detected only in fungi belonging to the family *Hypocreaceae*.

Fusarium lycopersici, the pathogen of a wilt of tomatoes in Europe and North America, produces dehydrofusaric acid as well as fusaric acid (28, 77). In culture filtrates of *F. vasinfectum*, the pathogen of a cosmopolitan wilt disease of cotton plants, only fusaric acid has so far been detected (28, 47, 52). *Nectria cinnabarina* (Tode) Fr., the pathogen of coral spot disease of trees and numerous other phanerogams, is known to produce fusaric acid only (25). *Gibberella fujikuroi* (= *Fusarium moniliforme* Sheld.), a pathogen of bakanaë disease of rice, maize, and sugar cane in Eastern Asia, produces both fusaric acid (28) and dehydrofusaric acid (75, 76). *Fusarium heterosporum* Nees, a ubiquitous saprophyte or nonspecific parasite of grasses, apples, ergot sclerotia, etc., produces fusaric acid and possibly dehydrofusaric acid. Since Yabuta, Kambe, and Hayashi (82) gave a high melting point for their fusaric acid preparation, it is possible that they were dealing with a mixture of fusaric acid and dehydrofusaric acid. Several Japanese and European authors have erroneously deemed this name to be a synonym of *Gibberella fujikuroi*, but the 2 fungi are not identical (4). *Fusarium orthoceras* Appel et Wr., a cosmopolitan saprophyte and a vascular and wilt pathogen of peas, potatoes, etc., produces fusaric acid. The identification of fusaric acid was made by Venkata Ram in Madras (communicated by letter). The presence of dehydrofusaric acid has not yet been established.

These 6 fungi differ markedly in their phytopathological aspects. *Fusarium lycopersici* and *F. vasinfectum* are pathogens of true wilt diseases, and their toxins can lead to the complete collapse of the host. The same is true for certain races of *F. orthoceras*. *Nectria cinnabarina*, in contrast, is a pronounced perthophyte that destroys the host tissues in its immediate vicinity but very seldom acts at a distance in the manner of wilt pathogens. *Gibberella fujikuroi* causes a quite different disease and acts either by means of direct necrosis or by stimulating adventitious growths similar to witches' brooms. *F. heterosporum* is only a semi-parasite and can live equally well as a saprophyte. Hence, according to our present knowledge, the capacity to synthesize fusaric acid is restricted to a single family of the fungi but, within this family, does not characterize either a given genus or species nor a given type of pathogen.

This wide range of formation of fusaric acid entails that in all the 6 named fungal species it must act with other metabolic products. Two examples, bakanaë disease of rice and wilt of tomato, will make this clearer.

The interaction of toxins in Gibberella fujikuroi.—This pathogen forms fusaric acid, dehydrofusaric acid, and 2 substances of the gibberellin group, namely, gibberellin A and gibberellic acid. The last 2 compounds possess the character of growth substances.

Gibberellin A is a highly aromatic nitrogen-free compound with the empirical formula $C_{22}H_{26}O_7$. In contrast to the auxins, it induces almost no cell division in the callus of Bryophyllum, does not suppress the development of axillary buds in peas, does not induce epinasty in tomato shoots, and is inactive in the ordinary Avena and Pisum tests (78). In contrast, it induces an elongation of the seedlings of germinating soybeans such that by the third day treated seedlings are approximately 2–4 cm longer than the controls. Further, their vitamin C content may show an excess of about one-fourth by the ninth

day (83, 84). In rice seedlings, gibberellin causes an elongation of the culms and leaves and later an increase in the dry weight (41, 83). Further, it stimulates amylase activity in germinating grains of barley, wheat, and rice (40).

Gibberellic acid, a derivative of gibberellin with the empirical formula $C_{19}H_{22}O_6$ (15), is formed mainly by the strains of *G. fujikuroi* occurring in the rice plant (4). It possesses physiological characters similar to those of gibberellin A but in more marked form; thus it causes lengthening of the internodes of many monocotyledons and dicotyledons and an increase of dry weight (3, 5, 6, 7, 56, 57, 58) but inhibits root development (5, 6, 7).

A mixture of gibberellic acid and of gibberellin A induces an elongation of internodes and the formation of flowers in *Hyoscyamus niger* L. (54, 55) and in 2 *Bryophyllum* species (8).

Thus, inside the rice plant, the action fields of 2 different groups of substances overlap. First, there are the true toxins of the fusaric acid group, which produce divergent effects according to their concentration (for example, at low concentrations they increase the water permeability of the protoplasts but reduce it at high concentrations (1, 2, 23, 26)). Second, there are the substances of the gibberellin group, which resemble growth substances, that also apparently injure the host tissues differently according to their concentration. Finally, the host also will influence this interplay of forces in that it will facilitate or inhibit the formation of one or another of these substances, according to its physiological status.

The varying interaction of these contrasting forces result in the labile and often contradictory course of the bakanaë disease: weak rice plants are arrested in their development and sometimes perish, whereas stronger individuals increase in size so that they stand out in the field because of their greater height (bakanaë effect (24; Fig. 415)).

The interaction of toxins in Fusarium lycopersici.—In this fungus the interplay of specific toxins is just as complex as in *G. fujikuroi* but perhaps less contradictory. Here, in addition to the fusaric acid and dehydrofusaric acid, the pathogen forms ethylene, lycomarasmin, vasinfuscarin, ethyl alcohol, and succinic acid.

Ethylene (C_2H_4), even at a concentration of about 10^{-7} M, induces epinasty of the petioles of young tomato plants; in stronger concentration, it causes a yellowing of the laminae (20).

In some ways lycomarasmin behaves similarly to fusaric acid and in some ways it differs, but the 2 induce similar symptoms in tomato plants (30).

Vasinfuscarin has not yet been examined in detail, but it is a toxin of enzymatic character that causes browning of the vascular bundles (33), thus inducing symptoms like those evoked by the nonspecific polyphenol oxidases (18, 19, 35, 79, 81).

The probable role of ethyl alcohol has already been discussed; its significance as a predisponent to disease is comparable with that of lycomarasmin and fusaric acid, at least at low concentrations such as are present at the start of pathogenesis.

Succinic acid, in biological concentrations, does not by itself induce any symptoms in tomato shoots; if applied at the same time, however, it heightens the toxicity of fusaric acid but not that of lycomarasmin (29).

Thus, in the case of *F. lycopersici*, the fusaric acid does not act alone on the host plant (as in our model experiments) but in conjunction with at least 5 or 6 other toxins and accompanying substances.

Phenocopies in tomato wilt.—The example of tomato wilt teaches us that in complex summation effects a similar disease picture, a phenocopy, may come into being even if important single components are replaced by other toxins. Thus, *F. lycopersici* is absent from South India, yet a wilt disease of tomato plants occurs there that is practically identical to that caused by *F. lycopersici*. It is caused by other Fusaria, including *F. lateritium* Nees and *F. scirpi* Lamb. et Fautr. (69). Up to the present, fusaric acid and lycomarasmin have not been detected in these 2 Fusaria; only enniatine A (amongst other yet undefined toxins) has been found (27, 32).

Enniatines are phytotoxic polypeptides of specific configuration (cyclic tetrapeptoids). The enniatine A under discussion here has the empirical formula $C_{24}H_{42}O_6N_2$ and a molecular weight of 455 (61, 64, 65, 66, 67, 68, 88); it is identical with lateritiin I (13, 14). Enniatine A is extremely poisonous to tomato tissues; the threshold for obvious damage is at a concentration of about 9.7×10^{-7} M (31).

Macroscopically, enniatine A first affects the stems of tomato plants, and at a dosage of only 15 mg/kg fresh weight produces a necrosis of the vascular bundles and lethal streaking in the cortical tissues. The other toxins and accompanying substances of these 2 Fusaria are still unknown. What is important in the present connection is that in a complex of toxins, such as is produced by phytopathogenic Fusaria, single toxins can be replaced by others without this showing in pathogenesis.

This low specificity of the summation effect is connected with the fact that a plant can respond to the toxins of a pathogen only within narrow limits; the more striking disease symptoms, such as the browning of vascular bundles or the necrosis of ground tissues, the stiffening of shoots or their wilt-collapse, are largely nonspecific and can be due to any number of causes. This condition, low specificity of response to stimulus, is well known in human medicine, e.g. nonspecific pain and fever.

The range of activity of fusaric acid.—The range of activity of fusaric acid is wide and nonspecific, corresponding to the biological diversity of the fungi that synthesize it.

Among the bacteria, growth of *Bacillus subtilis*, *Escherichia coli* (Mig.) Cast., *Bacillus megatherium* d By., and paratubercle bacilli is inhibited by a con-

centration of 10^{-4} M and that of *Streptococcus faecalis* Andr. et Hor., *Micrococcus pyogenes* var. *aureus*, *Pseudomonas aeruginosa*, and *Candida vulgaris* Berch. by a concentration of 10^{-3} M (28, 85).

Among the green algae, *Spirogyra nitida* (Dillw.) Link and *Haematococcus* sp. are very sensitive to fusaric acid; the water permeability of the former is impaired by fusaric acid even at a concentration of 5×10^{-8} M (2).

Among the fungi, the LD_{50} for the inhibition of the germination of chlamydospores of *Ustilago zeae* is about 1.5×10^{-4} M (48, 51), but the mycelial growth of *Corticium vagum* Berk. et Curt. is measurably inhibited only by a fusaric acid concentration of 10^{-3} M (70).

Among the phanerogams, dozens of different species of monocotyledons and dicotyledons react to fusaric acid; up to the present, we know of no phanerogamic test plant that is not injured by fusaric acid.

There is, therefore, a marked incongruity between the range of activity of fusaric acid and the host range of the pathogens that form it; thus, *F. lycopersici* normally attacks only tomato plants whereas one of its toxins, fusaric acid, injures higher plants of many species—plants never accessible to the fungus that produces it. The susceptibility or resistance of a host to the pathogen in question will, therefore, be determined by mechanisms other than its sensitivity or power of resistance to the toxins of this pathogen.

Within the wide phanerogamic host range, the sensitivity of the various host species to fusaric acid differs both quantitatively and qualitatively, although no generalizations can yet be made.

Table 2 gives for several host plants the quantities of fusaric acid needed to produce injuries of an intensity of 1.5 on a scale of 0 to 4, in which 0 indicates no injury and 4 indicates collapse of the plant. This injury threshold of 1.5 is termed the *dosis minima*. The method of assessment for tomato shoots will be described later; the scheme in question was adapted to the other hosts. Rye, maize, and peas react only mildly to fusaric acid; their *dosis minima* is about 1/10 that of beans or rice. Cotton plants react most sensitively, their *dosis minima* being about 1/100 that of rye, maize, or peas.

TABLE 2.—*The sensitivity of cuttings of various host species to fusaric acid (After Gäumann et al (28))*

Test plant	*Dosis minima* [a] mg/kg fresh weight
Rye, *Secale cereale* L.	1000–2000
Maize, *Zea mays* L.	1000–2000
Peas, *Pisum sativum* L.	1000–2000
Beans, *Phaseolus vulgaris* L.	100–200
Rice, *Oryza sativa* L.	100–200
Tomato shoots, *Lycopersicon esculentum* Mill.	150
Cotton, *Gossypium herbaceum* L.	10–20

[a] Minimum quantity that produces injuries of an intensity of 1.5 on a scale of 0 to 4, in which 0 indicates no injury and 4 indicates collapse of the plant.

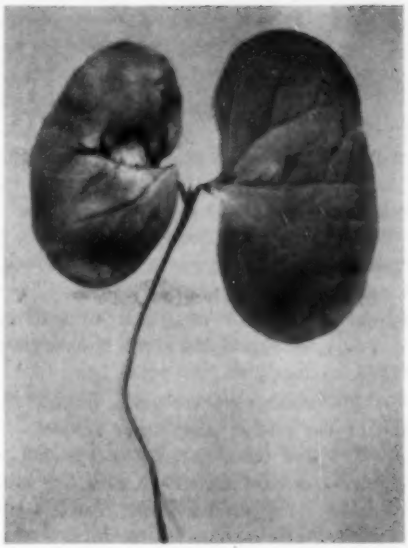


FIG. 11. Injury to a cotton seedling by fusaric acid. Toxin dosage 20 mg/kg fresh weight (×1). (After Gäumann et al (28)).

In addition to these quantitative degrees of toxin sensitivity, there also exist in the various host species considerable differences in the sensitivity of specific tissues, although these have not yet been worked out in detail. Thus, in young rye or rice plants the culm remains turgid after the administration of the *dosis minima*, and only the lowest leaf is injured. In contrast, the lowest part of the culm is the most sensitive part of the maize plant. In young pea and bean plants, however, the stem and the leaves are equally affected. Both stem and petioles of cotton seedlings are severely injured, but the primary leaves are initially affected only along their veins (Fig. 11).

These differences in symptom manifestation of fusaric acid injury allow one to surmise that not only are different amounts of fusaric acid required to evoke the same pathogenic effect in the various host species but also that the points of attack of fusaric acid are differently situated in different host species and are therefore tissue specific to a certain extent.

The scope of the range of activity of dehydrofusaric acid has not yet been thoroughly investigated, but it cannot be much less than that of fusaric acid. Like fusaric acid, dehydrofusaric acid injures tomato plants as well as the germinating chlamydospores of *Ustilago zeae*; in the leaves of tomato shoots its efficiency *ceteris paribus* is about that of pure fusaric acid, whereas in the stems of tomato shoots and in the Ustilago test it is only about one-half as efficient (75).

If, therefore, a culture filtrate is examined by means of the Ustilago test to determine its content of fusaric acid and dehydrofusaric acid, the values in the dehydrofusaric acid sector are too low. Precise figures can be obtained in this case only by quantitative chemical determination.

THE DETECTION OF FUSARIC ACID IN VIVO.—In rela-

tion to every metabolic product of a pathogen, the question arises of whether the pathogen produces it solely in artificial culture, in which case it is an artifact, or whether it is also synthesized in identical form inside the host.

In the case of fusaric acid, this question is very difficult to answer because the molecule is not stable (71). When tomato shoots take up 120 mg of labelled fusaric acid (i.e. synthetic fusaric acid with a ^{14}C-atom added to its carboxyl group) per kg of fresh weight, only 6.9 per cent is recognizable as intact fusaric acid after 48 hours, the remaining 93.1 per cent has "vanished," i.e. has in some way been transformed, fixed, or destroyed in the host tissues.

But if the tomato plants receive double the above dose, then at the end of the same period 3 times as much, i.e. 22.4 per cent, can be recognized as intact fusaric acid. It is probable that with the larger dose a threshold has been passed above which a proportion of the introduced fusaric acid no longer reacts with the host and therefore remains intact.

Human medicine finds a similar difficulty in the direct recognition of parasitogenic toxins *in vivo*. For example, it has not yet succeeded in demonstrating either the diphtheria or the tetanus toxins *in vivo*, either *en route* to the tissues they will injure or in these tissues themselves. It has to resort to the argument that the symptoms evoked in the host by toxins produced *in vitro* are the same as those that appear *in vivo* following infection.

The ideal demand that the chemical identity of a toxin produced *in vitro* and one produced *in vivo* should be proved incontrovertibly is clearly right in spite of all the technical difficulties; and it is through the researches of 2 independent groups of workers that the proof has been obtained that fusaric acid itself, or a substance very closely related to it, appears in the host plant as a consequence of infection.

In sap expressed from the roots, stems, and leaves of cotton plants 10 days after inoculation with *F. vasinfectum*, Lakshinarayanan and Subramanian (52) were able to establish chromatographically the presence in a copper complex of a substance identical with fusaric acid or at least very closely related to it. Using a similar method, Kalyanasundaram and Venkata Ram (47) showed that in young cotton plants inoculated with *F. vasinfectum* at least 17 mg of fusaric acid is present per kg of fresh weight.

Before and after these investigations, similar results were obtained by Kern and Sanwal (50) and by Kern and Kluepfel (49), who used isotope methods. When *F. lycopersici* was supplied with methyl-labelled ^{14}C glycine and the labelled fungus then inoculated into tomato plants, radioactive metabolic products were demonstrated in these plants after 36 hours. Thus, even during the first 36 hours after inoculation, i.e. at the start of the incubation period, the pathogen had built the ^{14}C atoms into metabolic products that were released in the vascular system of the host. In the course of the disease, the radioactivity was higher in the severely affected leaves than in those less affected, so that it may be concluded that some of these radioactive metabolic products are toxic to the host plants (50).

The experiments in which labelled mycelium and the isotope dilution technique are used to demonstrate the presence of fusaric acid directly may give faulty results because of the low radioactivity of the extracts. But the demonstration is successful if the tomato plants are inoculated with ordinary mycelium of *F. lycopersici* and are then maintained in an atmosphere of radioactive carbon dioxide. In this case, the mycelium progressing through the host takes up so much ^{14}C that radioactive fusaric acid or a compound closely related to it can be detected in the infected plants 10 days after inoculation (49).

Thus, it has been proved that fusaric acid is not merely an artifact of laboratory culture but that it is also formed in the host and therefore is a true "vivotoxin."

THE GROSS SYMPTOMS OF FUSARIC ACID INJURY IN TOMATO SHOOTS.—The gross symptoms of fusaric acid injury to be discussed here are, from the biological point of view, artifacts for 2 reasons. Firstly, fusaric acid under natural conditions never acts in isolation on the host but always in concert with other products of metabolism and with other toxins. Secondly, we use toxin concentrations (of the magnitude of 10^{-3} M) much higher than those normally found in biological systems in order to evoke a drastic manifestation of injury symptoms in tomato cuttings within 48 hours.

In consequence, the symptoms of fusaric acid injury in tomato shoots described here are scarcely ever seen in so marked a form in natural infections. Their importance lies essentially in the fact that, under such extreme conditions, one can ascertain in which tissues and under what circumstances fusaric acid and dehydrofusaric acid develop their pathogenic activity within tomato shoots.

The symptoms of fusaric acid injury.—If tomato cuttings are placed in a 10^{-3} M solution of fusaric acid, symptoms of injury appear first in the stems and soon after in the leaves.

In the stems, it is mainly the cortical tissues over the vascular bundles that are destroyed, and light gray-green sunken furrows appear on the stems and gradually spread to the petioles (Fig. 12). As previously mentioned, this furrow formation on the stem and petioles is common to fusaric acid and patulin toxicity (24; Fig. 406, 407). Next, the petioles start to bend downwards (epinasty) in the same way as Dimond and Waggoner (20) have described for ethylene injury. In contrast, the conducting strands in the stems and petioles are apparently uninjured and are not browned.

With stronger concentrations, the whole stem tissue is weakened so that the stem becomes threadlike (Fig. 11). This symptom may appear as early as 4 hours after the intake of toxin; 48 hours after intake, the development of symptoms is complete.

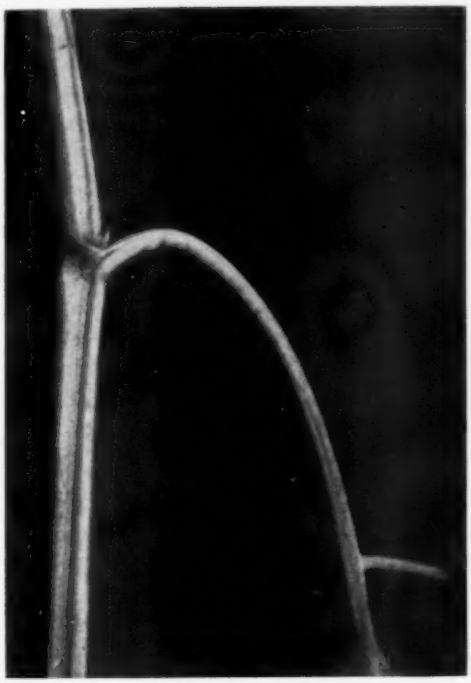


FIG. 12. Symptoms of fusaric acid injury on the stem of a tomato shoot (× ¾).

With dehydrofusaric acid, the effects on the stems and petioles of tomato shoots are less marked than with fusaric acid; usually they appear as a shrivelling of all the tissues.

In leaves of tomato shoots, the gross symptoms of fusaric acid poisoning appear somewhat later than on the stem. They are most abundant in the interveinal areas and appear as small, gray-green, sunken, transparent, usually irregular, necrotic spots strongly reminiscent of the syndrome of lycomarasmin injury (24; Fig. 402). Infrequently, necrosis extends along the whole length of the veins of the leaf, even before the interveinal areas show any symptoms (28; Fig. 4). Approximately 24 hours after toxin intake, the leaflets curl from both sides towards the middle and become limp and flaccid.

With dehydrofusaric acid, extensive yellow discolorations appear along the veins, as happens only exceptionally with fusaric acid, and the leaf margins die. Finally, as with fusaric acid, necrosis appears in the interveinal areas, and the whole plant becomes limp. Sometimes the leaf blades become irregularly curled.

Both fusaric acid and dehydrofusaric acid exert, therefore, a tissue-specific effect in tomato shoots. In the stems, they both produce visible injury only to the cortical tissues and not to the conducting tissues; in the leaves, they both injure in a characteristic manner only definite parts of the ground tissue, so that a skilled observer can distinguish their syndrome from that of other toxins.

The influence of toxin dosage on the development of symptoms.—To what extent does the damage to tomato shoots increase with increasing dosage of fusaric acid? To estimate the intensity of injury to stems, we use the following scale: *Grade 1*, softening and slight shrinkage around the cut end; *Grade 2*, the lower part of the stem (from the cut end to the insertion of the lowest leaf) more or less soft, collapsed and shrivelled, petioles uninjured; *Grade 3*, injury to the degree of Grade 2 on the petioles also, but only on their lower halves; *Grade 4*, stem and petioles soft for more than half their length, usually collapsed and shrivelled. For leaves, the scale is as follows: *Grade 1*, scattered, light, transparent, irregular spots on the laminae; *Grade 2*, spots numerous and often merging, leaf margins dry and slightly rolled; *Grade 3*, spots as in Grade 2 but more marked, margins more heavily rolled, leaf flaccid; *Grade 4*, the entire leaf completely wilted, dark green, and rolled.

Table 3 shows the usual asymptotic course for the injuries of both stem and leaves. The *dosis minima* (by which an average injury rating of 1.5 will be produced) is about 150 mg of toxin per kg of fresh weight; the sublethal dose (Grade 3 injury) is about 300 mg toxin per kg of fresh weight.

The rapid increase in the rating for leaf symptoms from 1.7 to 2.8 when the toxin dosage is raised from 225 to 300 mg per kg of fresh weight cannot be attributed solely to the direct effect of the toxin but arises partly from damage to the stem. Thus, if the stem of a tomato shoot is severely injured by toxin (intensity higher than Grade 2), its function is impaired or destroyed. In such cases, leaf damage has a dual origin: 1) direct injury to the leaves by the toxin itself and 2) indirect injury to the leaves resulting from functional disturbance in the stem.

The influence of the degree of dissociation of the fusaric acid molecule on its pathogenicity.—What influence on the toxicity of the fusaric acid molecule has the reaction of the tissue substrate in which it must act *in vivo?* In other words, can the tissue-specific action of fusaric acid mentioned above be explained in part by the differential acidities of these tissues?

Pure fusaric acid has a pH of 4.3. The pH of the fusaric acid solutions were adjusted to the values shown in Table 4, and it was assumed that they would remain at these pH values inside the very slightly buffered vessels and cell walls of the ground and cortical tissues. Tomato cuttings were placed in the

TABLE 3.—*Symptom production by different toxic dosages of fusaric acid to young tomato shoots (After Gäumann et al (28))*

Toxin dosage [a] in mg/kg fresh weight	Average injury rating [b]	
	Stem	Leaves
75	1.1	0.5
150	2.0	1.5
225	2.6	1.7
300	3.0	2.8

[a] Concentration of toxin solution, 2.5×10^{-3} M. Reaction of toxin solution, pH 4.3.

[b] Injuries on a scale of 0 to 4, in which 0 indicates no injury and 4 indicates collapse of the plant.

TABLE 4.—*Symptom intensity in tomato shoots placed in solutions of fusaric acid adjusted to various pH values (After Gäumann et al (28))*

pH of toxin solution[a]	Average injury rating[b]	
	Stem	Leaves
4.3	1.75 ± 0.60	0.93 ± 0.38
5.0	1.33 ± 0.49	0.65 ± 0.48
5.6	1.0 ± 0.37	0.77 ± 0.53
6.2	0	1.37 ± 0.44
6.8	0	1.63 ± 0.68

[a] Toxin dosage 150 mg per kg fresh weight in a concentration of 2.5×10^{-3}M.

[b] Mean value and standard deviation for each group of 15 experimental plants. Ratings are on a scale of 0 to 4, in which 0 indicates no injury and 4 indicates collapse of the plant.

toxin solution until they had taken up the *dosis minima*, after which they were returned to tap water.

Fusaric acid at its natural pH of 4.3 caused maximum damage to the cortical tissues of the stem (Table 4). With increasing alkalinity, the intensity of injury declined and disappeared entirely at about pH 6.0.

In contrast, the leaves of tomato shoots reacted less strongly to pH changes in the toxin solution. Table 4 shows that no statistically significant alteration of symptom development can be recognized from pH 4.3 to pH 5.6. Only in the range of pH 6.2 and above did injury to the tomato leaves clearly increase as pH was increased; in other experiments, the intensity of damage increased at pH of 7.6 to an average value of 2.6. Here also, therefore, the critical point must be about pH 6.0.

When the tomato shoots received only the *dosis minima*, symptom development took opposite courses in stem and leaves as the pH of the solution was varied: in the cortical tissues of the stem, symptoms disappeared when the reaction of the toxin solution was increased above pH 6.0, whereas symptoms in leaves increased in intensity (Table 4).

This state of affairs may be accounted for in the following way (28). Fusaric acid is a weak monobasic acid, even weaker than acetic acid; the dissociation constant of the former is 10^{-6}, and that of the latter is 1.8×10^{-5} M. The degree of dissociation of fusaric acid can be calculated as a function of pH as indicated in Table 5.

As a rule, negatively charged particles (acid ions) have difficulty in crossing the plasma membrane, whereas neutral or near-neutral particles like fusaric acid molecules meet with less resistance. The end result, the quantity of fusaric acid particles that succeed in passing from the cell walls, through the plasma membrane, and into the cells where they exert their specific action therefore depends upon their ability to enter the cell. This in turn is determined by dissociation relationships.

TABLE 5.—*Calculated dissociation of fusaric acid at various pH values*

pH	Degree of dissociation
	%
4.3	2 (approximate)
5.6	30 (approximate)
6.0	50
6.8	86 (approximate)
8.7	100

At a reaction below pH 6.0, i.e. in a comparatively slightly dissociated state, a proportionately large number of fusaric acid molecules move from the cell walls through the plasma membrane into the cell. Hence, they surmount the critical concentration threshold and cause obvious injuries in both the ground tissue and cortical cells of the stem, which are more or less isolated from the transpiration stream.

Because of this consumption of fusaric acid by the stem tissues, a proportion of the available toxin that would otherwise have reached the leaves is utilized. In treating the tomato shoots with 150 mg per kg fresh weight, we are giving approximately only the *dosis minima*. The toxin dosage needed to evoke marked symptoms of injury in tomato shoots within 48 hours is, therefore, somewhere near the minimum. In consequence, the fusaric acid "demands" of the stem and of the leaves are in competition: the fusaric acid that is utilized in the ground tissue cells and cortical cells of the stem is not available for use in the leaves. Therefore if symptoms appear in the stem, there must be a certain suppression of symptoms in the leaves. Table 4 shows this to be the case.

In contrast, this competition between stem and leaves does not take place if the tomato shoots are given an excess of fusaric acid, e.g. 225 mg per kg of fresh weight. Accordingly, the leaf symptoms are not influenced by the presence or absence of stem symptoms but appear with equal severity throughout the entire pH range.

This competition between the fusaric acid consumption of stem and leaves undoubtedly plays an important role *in vivo*, where the amount of fusaric acid available is much less than in our laboratory experiments.

At reactions above pH 6.0, i.e. in a state of high dissociation (over 50 per cent), only comparatively few fusaric acid molecules succeed in penetrating directly from the cell walls through the plasma membrane into the cell. Because of this slight consumption of fusaric acid by the stem tissues, only a relatively small amount of toxin is utilized and a correspondingly larger proportion is available for leaf activity. Thus it becomes understandable why injury to the foliage is clearly greater in the pH range in which stem symptoms are absent.

No exact study has yet been made of the pH conditions inside young tomato shoots, but this has been done for *Solanum dulcamara* L. and *Solanum tuberosum* L. (74). We must, therefore, draw our conclusions by analogy.

In these 2 species of *Solanum* the pH in the various tissues or plant parts is as follows: xylem, 4.0–4.4;

central pith, 5.9; endodermis and cortical tissues, 5.6–5.9; epidermis, 4.8–5.2 (sometimes 5.6); hairs, 4.8–5.2 (sometimes over 6.2); and potato tubers, 6.2. These values might be expected to be approximately the same as for young tomato shoots. Hence, the ability of fusaric acid to enter the cell will differ markedly in the vessels and in the ground and cortical tissues of tomato shoots.

Inside the vessels (pH 4.0–4.4), the fusaric acid can move essentially undissociated; this is favoured by the circumstance that the constituents of the xylem walls are negatively charged, a condition that would accelerate the upward movement of fusaric acid molecules (see Charles (9) for data on penicillin, which is similarly acid). Fusaric acid is also correspondingly unhindered in its penetration of the cells of the vascular bundle parenchyma. Thus it apparently finds its optimal conditions of activity in the vessels and in the vascular bundle parenchyma and produces there the maximum injury of which it is capable, although this damage is not as yet apparent to the eye.

This coincidence of the optimal pH for the activity of fusaric acid being within the actual pH range found in the xylem must be decisive for the pathogenesis of tomato wilt. In the course of the disease, fusaric acid is the first toxin (besides ethylene) to surmount the threshold of permanent cell injury and so to prepare the way for other toxic substances (toxins and enzymes). Because it finds its conditions of optimal activity in the vascular bundles (even if it produces no visible injuries there), it is no accident that tomato wilt is primarily a vascular bundle disease.

In the ground and cortical tissues of tomato shoots (about pH 6.0), almost 50 per cent of the fusaric acid molecules are dissociated; therefore, they permeate the tissue only with difficulty.

It will at once be evident that these considerations regarding pH concern only some of the conditions determining the pathogenic activity of fusaric acid. Inside the host, matters are infinitely more complicated. We hope to return to these questions in a further paper on the mechanism of fusaric acid injury.

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LITERATURE CITED

1. Ahmet, H. 1933. Untersuchungen über Tracheomykosen. Phytopathol. Z. **6:** 49-101.
2. Bachmann, E. 1956. Der Einfluss von Fusarinsäure auf die Wasserpermeabilität von pflanzlichen Protoplasten. Phytopathol. Z. **27:** 255-288.
3. Barton, L. V. 1956. Growth response of physiological dwarfs of Malus Arnoldiana Sarg. to gibberellic acid. Contrib. Boyce Thompson Inst. **18:** 311-317.
4. Borrow, A., P. W. Brian, V. E. Chester, P. J. Curtis, H. G. Hemming, C. Henehan, E. G. Jeffreys, P. B. Lloyd, I. S. Nixon, G. L. F. Norris, and M. Radley. 1955. Gibberellic acid, a metabolic product of the fungus Gibberella Fujikuroi: some observations on its production and isolation. J. Sci. Food Agr. **6:** 340-348.
5. Brian, P. W., G. W. Elson, H. G. Hemming, and M. Radley. 1954. The plant-growth-promoting properties of gibberellic acid, a metabolic product of the fungus Gibberella Fujikuroi. J. Sci. Food Agr. **5:** 602-612.
6. Brian, P. W., and H. G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. Physiol. Plantarum **8:** 669-681.
7. Brian, P. W., H. G. Hemming, and M. Radley. 1955. A physiological comparison of gibberellic acid with some auxins. Physiol. Plantarum **8:** 899-912.
8. Bünsow, R., and R. Harder. 1956. Blütenbildung von Bryophyllum durch Gibberellin. Naturwissenschaften **43:** 479-480.
9. Charles, A. 1953. Uptake of dyes into cut leaves. Nature **171:** 435-436.
10. Chester, C. G. C., and G. N. Rolinson. 1951. Trace elements and streptomycin production. J. Gen. Microbiol. **5:** 559-565.
11. Clauson-Kaas, N., P. A. Plattner, and E. Gäumann. 1944. Ueber ein welkeerzeugendes Stoffwechselprodukt von Fusarium lycopersici Sacc. Ber. schweiz. botan. Ges. **54:** 523-528.
12. Clayton, E. E. 1923. The relation of soil moisture to the Fusarium wilt of the tomato. Am. J. Botany **10:** 133-147.
13. Cook, A. H., S. F. Cox, and T. H. Farmer. 1948. Antibiotics produced by fungi and a new phenomenon in optical resolution. Nature **162:** 4106, 61 p.
14. Cook, A. H., S. F. Cox, T. H. Farmer, and M. S. Lacey. 1947. Production of antibiotics by Fusaria. Nature **160:** 31-32.
15. Cross, B. E. 1954. Gibberellic acid. I. J. Chem. Soc. **1954:** 4670-4676.
16. Crowdy, S. H., D. Gardner, J. F. Grove, and D. Pramer. 1955. The translocation of antibiotics in higher plants. I. Isolation of griseofulvin and chloramphenicol from plant tissue. J. Exp. Botan. **6:** 371-383.
17. Davis, D. 1954. The use of intergeneric grafts to demonstrate toxins in the Fusarium wilt disease of tomato. Am. J. Botany **41:** 395-398.
18. Davis, D., and A. E. Dimond. 1954. The source and role of phenols in Fusarium wilt symptoms. Phytopathology **44:** 485-486.
19. Davis, D., P. E. Waggoner, and A. E. Dimond. 1953. Conjugated phenols in the Fusarium wilt syndrome. Nature **172:** 959.
20. Dimond, A. E., and P. E. Waggoner. 1953. The cause of epinastic symptoms in Fusarium wilt of tomatoes. Phytopathology **43:** 663-669.
21. Ettlinger, L. 1946. Zur kulturellen Gewinnung von Penicillin. Ber. schweiz. botan. Ges. **56:** 681-695.
22. Flück, V., and K. H. Richle. 1955. Papierchromatographische Untersuchungen über den Aminosäuren-Stoffwechsel von Fusarium lycopersici Sacc. Phytopathol. Z. **24:** 455-461.
23. Freeman, V. J. 1951. Studies on the virulence of bacteriophage-infected strains of Corynebacterium diphtheriae. J. Bacteriol. **61:** 675-688.
24. Gäumann, E. 1951. Pflanzliche Infektionslehre. Ed. 2. Birkhäuser. Basle. 681 p.
25. Gäumann, E. 1954. Toxins and plant diseases. Endeavour **13:** 198-204.
26. Gäumann, E., and W. Loeffler. 1957. Ueber die Wirkung von Fusarinsäure auf die Wasserpermeabilität der Markzellen von Tomatenpflanzen. Phytopathol. Z. **28:** 319-328.
27. Gäumann, E., St. Naef-Roth, and L. Ettlinger. 1950. Zur Gewinnung von Enniatinen aus dem Myzel verschiedener Fusarien. Phytopathol. Z. **16:** 289-299.
28. Gäumann, E., St. Naef-Roth, and H. Kobel. 1952.

Ueber Fusarinsäure, ein zweites Welketoxin des Fusarium lycopersici Sacc. Phytopathol. Z. **20:** 1-38.

29. Gäumann, E., St. Naef-Roth, and H. Kobel. 1952. Les synergismes chez les toxines de Fusarium lycopersici Sacc. Compt. Rend. **234:** 276-278.
30. Gäumann, E., St. Naef-Roth, and G. Miescher. 1950. Untersuchungen über das Lycomarasmin. Phytopathol. Z. **16:** 257-288.
31. Gäumann, E., St. Naef-Roth, P. Reusser, and A. Ammann. 1952. Ueber den Einfluss einiger Welketoxine und Antibiotica auf die osmotischen Eigenschaften pflanzlicher Zellen. Phytopathol. Z. **91:** 160-220.
32. Gäumann, E., St. Roth, L. Ettlinger, P. A. Plattner, and U. Nager. 1947. Enniatin, ein neues, gegen Mykobakterien wirksames Antibioticum. Experientia **3:** 202.
33. Gäumann, E., C. Stoll, and H. Kern. 1953. Ueber Vasinfuscarin, ein drittes Welketoxin des Fusarium lycopersici Sacc. Phytopathol. Z. **20:** 245-247.
34. Gothoskar, S. S., R. P. Scheffer, M. A. Stahmann, and J. C. Walker. 1955. Further studies on the nature of Fusarium resistance in tomato. Phytopathology **45:** 303-307.
35. Gothoskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1953. The role of pectic enzymes in Fusarium wilt of tomato. Phytopathology **43:** 535-536.
36. Gottlieb, D. 1944. The mechanism of wilting caused by Fusarium bulbigenum var. lycopersici. Phytopathology **34:** 41-59.
37. Groman, N. B. 1953. The relation of bacteriophage to the change of Corynebacterium diphtheriae from avirulence to virulence. Science **117:** 297-299.
38. Hardegger, E., and E. Nikles 1956. Fusarinsäure-[carboxyl-^{14}C] und quaternäre Derivate der Fusarinsäure. Helv. Chim. Acta **39:** 223-229.
39. Hardegger, E., and E. Nikles. 1956. Untersuchungen in der Pyridin-Reihe; ein neuer Weg zur Fusarinsäure. Helv. Chim. Acta **39:** 505-513.
40. Hayashi, T. 1940. Effect of gibberellin on the activity of amylase in germinated cereal grains. J. Agr. Chem. Soc. Japan **16:** 531-538. (Chem. Abstr. **35:** 772. 1941).
41. Hayashi, T., Y. Takijima, and Y. Murakami. 1953. The physiological action of gibberellin IV. J. Agr. Chem. Soc. Japan **27:** 672-675.
42. Heinze, P. H., and C. F. Andrus. 1945. Apparent localization of Fusarium wilt resistance in the Pan America tomato. Am. J. Botany **32:** 62-66.
43. Irving, G. W. 1947. The significance of tomatin in plant and animal disease. J. Wash. Acad. Sci. **37:** 293-296.
44. Irving, G. W., T. D. Fontaine, and S. P. Doolittle. 1945. Lycopersicin, a fungostatic agent from the tomato plant. Science **102:** 9-11.
45. Irving, G. W., T. D. Fontaine, and S. P. Doolittle. 1946. Partial antobiotic spectrum of tomatin, an antibiotic agent from the tomato plant. J. Bacteriol. **52:** 601-607.
46. Kalyanasundaram, R., and L. Saraswathi-Devi. 1955. Zinc in the metabolism of Fusarium vasinfectum Atk. Nature **175:** 945.
47. Kalyanasundaram, R., and C. S. Venkata Ram. 1956. Production and systemic translocation of fusaric acid in Fusarium-infected cotton plants. J. Indian Botan. Soc. **35:** 7-10.
48. Kern, H. 1952. Ueber die Beziehungen zwischen dem Alkaloidgehalt verschiedener Tomatensorten und ihrer Widerstandsfähigkeit gegen das Fusarium lycopersici Sacc. Phytopathol. Z. **19:** 351-382.
49. Kern, H., and D. Kluepfel. 1956. Die Bildung von Fusarinsäure durch Fusarium lycopersici in vivo. Experientia **12:** 181-182.
50. Kern, H., and B. D. Sanwal. 1954. Untersuchungen über den Stoffwechsel von Fusarium lycopersici mit Hilfe von radioaktivem Kohlenstoff. Phytopathol. Z. **22:** 449-453.
51. Kobel, F. 1951. Untersuchungen über toxische Stoffwechselprodukte von Nectria cinnabarina (Tode) Fr. Phytopathol. Z. **18:** 157-195.
52. Lakshminarayanan, K., and D. Subramanian. 1955. Is fusaric acid a vivotoxin? Nature **176:** 697-698.
53. Landolt, E. 1952. Ueber Welkestoffbildung bei Fusarium culmorum (W. G. Sm.) Sacc. Phytopathol. Z. **19:** 126-128.
54. Lang, A. 1956. Stem elongation in a rosette plant, induced by gibberellic acid. Naturwissenschaften **43:** 257-258.
55. Lang, A. 1956. Induction of flower formation in biennial Hyoscyamus by treatment with gibberellin. Naturwissenschaften **43:** 284-285.
56. Lona, F. 1956. L'azione dell'acido gibberellico sull'accrescimento caulinare di talune piante erbacee in condizioni esterne controllate. Nuovo giorn. botan. ital. **63:** 61-76.
57. Lona, F., and A. Bocchi. 1956. Caratteristiche d'accrescimento e sviluppo di alcune razze invernali di cereali trattate con acido gibberellico. Riv. intern. agr. July, 1956. p. 44-47.
58. Lona, F., and A. Bocchi. 1956. La distensione caulinare nella canapa incrementata dall'acido gibberellico. Riv. intern. agr. August, 1956. p. 58-60.
59. Luz, G. 1934. Ueber den Stoffwechsel von Fusarium lycopersici und Fusarium lini. Phytopathol. Z. **7:** 585-638.
60. Miescher, G. 1950. Ueber die Wirkungsweise von Patulin auf höhere Pflanzen, insbesondere auf Solanum Lycopersicum L. Phytopathol. Z. **16:** 369-397.
61. Nager, U. 1948. Ueber die Chemie der Enniatine. Thesis. Swiss Federal Inst. Technology. Zurich. 42 p.
62. Nakashima, T. 1955. Studies on fusaric acid and analogous compounds I. Synthesis of 3-sec-butylpyridine. J. Pharm. Soc. Japan. **75:** 1010-1011.
63. Plattner, P. A., W. Keller, and A. Boller. 1954. Konstitution und Synthese der Fusarinsäure. Helv. Chim. Acta **37:** 1379-1392.
64. Plattner, P. A., and U. Nager. 1947. Ueber die Chemie des Enniatins. Experientia **3:** 325-326.
65. Plattner, P. A., and U. Nager. 1948. Ueber die Konstitution von Enniatin B. Helv. Chim. Acta **31:** 665-671.
66. Plattner, P. A., and U. Nager. 1948. Ueber die Konstitution von Enniatin A. Helv. Chim. Acta **31:** 2192-2203.
67. Plattner, P. A., and U. Nager. 1948. Analyse und Charakterisierung der Enniatine. Ueber das Verhalten von N-Methyl-aminosäuren im Papierchromatogramm. Helv. Chim. Acta **31:** 2203-2209.
68. Plattner, P. A., U. Nager, and A. Boller. 1948. Welkestoffe und Antibiotika VII. Ueber die Isolierung neuartiger Antibiotika aus Fusarien. Helv. Chim. Acta **31:** 594-602.
69. Prasanna Varma, R. 1954. Soil conditions and root diseases. X. The tomato wilt Fusaria. J. Indian Botan. Soc. **33:** 43-72.
70. Reusser, P., and St. Naef-Roth. 1956. Ueber den Einfluss der Fusarinsäure auf den Gaswechsel von Saccharomyces cerevisiae Hans. Phytopathol. Z. **26:** 273-296.
71. Sanwal, B. D. 1956. Investigations on the metabolism of Fusarium lycopersici Sacc. with the aid of radioactive carbon. Phytopathol. Z. **25:** 333-384.
72. Scheffer, R. P., and J. C. Walker. 1954. Distribution and nature of Fusarium resistance in the tomato plant. Phytopathology **44:** 94-101.
73. Sherwood, E. C. 1923. Hydrogen ion concentration as related to the Fusarium wilt of tomato seedlings.

Am. J. Botany **10**: 537-553.
74. SMALL, J. 1955. The pH of plant cells. Springer, Vienna. 116 p.
75. STOLL, C. 1954. Ueber Stoffwechsel und biologisch wirksame Stoffe von Gibberella Fujikuroi (Saw.) Woll., dem Erreger der Bakanaëkrankheit. Phytopathol. Z. **22**: 233-274.
76. STOLL, C., AND J. RENZ. 1957. Ueber den Fusarinsäure- und Dehydrofusarinsäurestoffwechsel von Gibberella Fujikuroi (Saw.) Woll. Phytopathol. Z. (In press).
77. STOLL, C., J. RENZ, AND E. GÄUMANN. 1956. Ueber die Bildung verschiedener Fusarinsäuren durch das Fusarium lycopersici Sacc. in saprophytischer Kultur. Phytopathol. Z. (In press).
78. SUMIKI, Y. 1952. The physiological action of gibberellin III. J. Agr. Chem. Soc. Japan **26**: 393-397.
79. WAGGONER, P. E., AND A. E. DIMOND. 1955. Production and role of extracellular pectic enzymes of Fusarium oxysporum f. lycopersici. Phytopathology **45**: 79-87.
80. WHITE, R. P. 1927. Studies on tomato wilt caused by Fusarium lycopersici Sacc. J. Agr. Research **34**: 197-239.
81. WINSTEAD, N. N., AND J. C. WALKER. 1954. Production of vascular browning by metabolites from several pathogens. Phytopathology **44**: 153-158.
82. YABUTA, T., K. KAMBE, AND T. HAYASHI. 1934. Biochemistry of the bakanaë-fungus. I. Fusarinic acid, a new product of the bakanaë-fungus. J. Agr. Chem. Soc. Japan. **10**: 1059-1068. (Chem. Abstr. **29**: 1132. 1937).
83. YABUTA, T., Y. SUMIKI, K. FUKUNAGA, AND M. HORIUCHI. 1951. Chemical composition of rice seedlings treated with gibberellin. J. Agr. Chem. Soc. Japan **24**: 396-397. (Chem. Abstr. **46**: 5143, 1952).
84. YABUTA, T., Y. SUMIKI, N. MURAYAMA, AND K. SUZUKI. 1941. The effects of gibberellin on the components of soybean malt. J. Agr. Chem. Soc. Japan **17**: 527-528. (Chem. Abstr. **44**: 10814, 1950).
85. YASUE, Y. 1949. Studies on the antibacterial action of fusaric acid, a metabolic product of the causative mould of the "Bakanaë" disease of rice plants. J. Antibiotics **2**: 262-264.
86. ZÄHNER, H. 1954. Die Bestimmung der Fusarinsäure mit Hilfe der Papierchromatographie. Phytopathol. Z. **22**: 227-228.
87. ZÄHNER, H. 1955. Welketoxine. In Linskens, H.F. ed., Papierchromatographie in der Botanik. Springer, Berlin. p. 194-197.
88. ZEHNDER, K. 1951. Ueber die Konstitution der N. Methyl-aminosäuren aus Enniatin A und B. Thesis. Swiss Federal Inst. Technology, Zurich. No. 1983, 96 p.

RELATION OF FUNGICIDE AND MITICIDE TREATMENTS TO WINTER INJURY AND SPRING BLACKSPOT DEVELOPMENT ON ROSES[1]

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SUMMARY

In connection with field trials of 29 fungicide-miticide dust combinations on 5 varieties of roses for the control of blackspot (caused by *Diplocarpon rosae* Wolf) and of spider mites (*Tetranychus telarius* L.), a close correlation was observed between blackspot control one year and early flower production the following year. The best blackspot control one year resulted in the best flower yield the beginning of the following season. The carry-over of the blackspot fungus on rose plants was least where fungicidal treatments against blackspot were effective the previous year and greatest where control was ineffective. In general, winter killing of rose canes was greatest in plots that had severe blackspot the previous year. Greater differences in amount of winter killing between treatments was obtained with the Golden Masterpiece variety than with the Red Radiance variety; the former variety was readily defoliated by blackspot. There was evidence also that winter killing was less in plots that had good mite control the previous year than in plots where mite control was poor.

In 1954, tests were initiated to determine compatibilities of various fungicide-miticide combinations and their effectiveness on 5 varieties of roses in 120 field plots at Beltsville, Maryland. These and later tests on other plots and with the same or other varieties provided much information on control of blackspot (caused by *Diplocarpon rosae* Wolf) and of two-spotted spider mite (*Tetranychus telarius* L.) (6,7,11) as well as on compatibilities of combinations (7,10). Flower yields were directly related to effectiveness of the control of blackspot and of spider mites. Also the fungicide and miticide treatments made one year influenced winter killing of rose canes and blackspot development the following year. This relation of fungicide and miticide treatments to the overwintering of the blackspot organism was reported briefly in an abstract (8), and the details about that, as well as the relation to winter injury, are reported here.

MATERIALS AND METHODS.—The plot design and procedures in these tests, described in detail previously (7,11), are outlined briefly here. The 5 varieties of roses used were Red Radiance, mite susceptible and blackspot tolerant; Golden Masterpiece and Chief Seattle, both blackspot susceptible and readily defoliated by blackspot; Peace, blackspot tolerant; and Christopher Stone, mildew susceptible. Four plants of each of the first 3 varieties and 2 of each of the last 2 were planted in each of 120 plots in the spring of 1954. Twenty-nine different fungicide-miticide dust

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combinations were compared with an untreated check in each of 4 replicates. The fungicidal treatments used were 3.4 per cent copper from basic copper sulfate (Tennessee Copper 34); 3.4 per cent copper and 25 per cent sulfur; 7.6 per cent ferbam (ferric dimethyldithiocarbamate) and 25 per cent sulfur; 5 per cent captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide); and 1 per cent Karathane (2-(1-methylehptyl)-4,6-dinitrophenyl crotonate and isomers), which is ineffective against blackspot but was included for powdery mildew control. A no-fungicide treatment and the fungicidal treatments listed were used alone or in combination with each of 3 miticides: 1.5 per cent Aramite (product containing 2-(*p-tert*-butylphenoxy) isopropyl-1-methylethyl 2-chloroethyl-sulfite), 4 per cent malathion (S-(1,2-dicarbethoxy-ethyl)-O,O-dimethyl dithiophosphate), and 1.5 per-cent ovex (product containing *p*-chlorophenyl-*p*-chloro-benzenesulfonate). Also, 25 per cent sulfur, 7.6 per-cent ferbam, 6.5 per cent zineb (zinc ethylenebis[dithiocarbamate]), and 5 per cent captan were each used in a mixture of Karathane and Aramite. In addition, zineb was used alone and in combination with Aramite. Five per cent DDT (commercially available dichlorodiphenyl-trichloroethane, the principal constituent of which is 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane), and 1 per cent lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) were used in all dust mixtures. Weekly dust applications were made during the growing seasons of 1954 and 1955, each plot receiving the same dust mixtures both years. Eighteen applications were made in 1954 and 16 in 1955. Applications were begun both years either in late May or early June and terminated at the end of September. Because of excessive rainfall, no applications were made during an 18-day period in August, 1955.

To ensure uniform infection, the plants were sprayed with suspensions of Diplocarpon spores during 2 periods of wet weather in July, 1954. The plots were rated visually for blackspot severity, and spider mite counts were made several times during each growing season (7). The blackspot ratings made in September, 1954, were made prior to the final dust treatment of the 1954 season, and those made in June, 1955, were made too soon to be affected by dust treatments.

Yield data were obtained by cutting the roses twice each week and recording the number and weight of flowers of each variety and in each treatment. Winter killing was determined by estimating the percentage of dead wood in each variety in each plot in March, 1955, and June, 1956.

RESULTS.—During the 2 years of tests, there were marked differences in yields of plants treated with different dust mixtures; these yield differences, in both number and weight of flowers, were attributed to differences in the control of blackspot and of spider mites (7, 11). Furthermore, blackspot control one season was reflected in the amount of blackspot that developed at the beginning of the following season. The results with Golden Masterpiece roses are given in Table 1. Plants treated with copper, Karathane, or no fungicide during 1954 were severely affected by blackspot by September, whereas plots receiving zineb, sulfur-copper, or sulfur-ferbam were only moderately affected, and those receiving captan were intermediately affected. In 1955, treatments were begun in early June; thus, blackspot appearing on the plants in June reflected the previous year's treatment. More blackspot was present in the plots treated with Karathane or no fungicide, than was present in other plots. Plots treated with zineb, sulfur plus copper, captan, and sulfur plus ferbam had significently less blackspot than did plots receiving no fungicide or Karathane. Plots dusted with copper tended to have less blackspot than did those not treated. Similar results were obtained with Chief Seattle and Red Radiance roses, but the differences between treatments were not so great with the last variety. Peace and Christopher Stone roses were not rated for blackspot severity.

TABLE 1.—*Blackspot severity on Golden Masterpiece roses September, 1954, and June, 1955, as affected by fungicidal treatments in 1954*

Fungicidal treatment	Blackspot severity [a]	
	September, 1954	June, 1955 [b]
None	83.4	54.7
Karathane	84.5	54.1
Copper	86.1	40.6
Captan	69.8	35.6
Sulfur-ferbam	44.2	27.2
Sulfur-copper	40.3	31.8
Zineb	41.9	25.0
LSD at odds of 19:1	6.6	15.0

[a] Based on a relative scale, with maximum blackspot = 100 and none = 0.

[b] The 1955 dust applications were begun May 24, too late to affect these ratings.

Flower production by Golden Masterpiece roses in May and June, 1955, also reflected the 1954 treatments (Table 2). Fewer flowers with less weight were produced in plots on which Karathane, copper, or no fungicide was used the previous year than in plots where sulfur plus copper, sulfur plus ferbam, or zineb

TABLE 2.—*Yield of Golden Masterpiece roses in May and June, 1955, following fungicide treatments in 1954* [a]

Fungicidal treatment	Number of flowers	Total weight of flowers (kilograms)	Weight per flower (grams)	
			May	June
None	492	5.1	11.3	9.8
Karathane	451	4.6	11.2	9.4
Copper	519	5.7	12.1	10.0
Captan	818	9.5	12.2	11.0
Sulfur-ferbam	1321	15.1	11.9	10.9
Sulfur-copper	1479	17.2	11.7	11.3
Zineb [b]	1323	17.0	12.6	10.7
LSD at odds of 19:1	160	1.9		

[a] The 1955 dust applications were begun May 24, too late to affect these numbers and weights.

[b] Not included in the statistical analyses.

TABLE 3.—*Winter killing of Golden Masterpiece roses following treatment with different fungicides that gave various degrees of blackspot control*

Fungicidal treatment during 1954 and 1955 growing seasons	Blackspot rating [a] (September, 1954)	Wood winter killed (March, 1955)	Blackspot rating [a] (July, 1955)	Wood winter killed (1956)
		%		%
None	83.4	84.2	69.5	77.1
Karathane	84.5	82.5	64.2	63.5
Copper	86.1	72.1	47.5	40.5
Captan	69.8	42.1	55.4	35.3
Sulfur-ferbam	44.2	14.2	33.6	30.0
Sulfur-copper	40.3	15.0	40.4	41.8
Zineb	41.9	16.3	32.3	33.6
LSD at odds of 19:1	6.6		8.0	

[a] Based on a relative scale with maximum blackspot = 100 and none = 0.

was used. Flower production in the captan-treated plots was intermediate; statistically, the yields were significantly better than those of the no-fungicide or the Karathane plots. The total weight of flowers harvested was related to treatment and to blackspot control. Surprisingly, the average weight per flower in May was about the same regardless of treatment, whereas in June the average weight was related to treatment.

Severe winter killing occurred during both winters of the tests, and a large percentage of wood was killed back by the cold. This killing may have been accentuated by the heavy sawdust mulch used in the plots, as John L. Creech (unpublished information) has demonstrated that azaleas mulched in late fall are more subject to winter injury than those not mulched. The 5 rose varieties in these plots varied considerably in susceptibility to winter injury. In the spring of 1955, 83.2 per cent of the wood of Christopher Stone, 72.7 per cent of that of Chief Seattle, 48.2 per cent of that of Golden Masterpiece, 44.2 per cent of that of Red Radiance, and 24.4 per cent of that of Peace was winter killed.

In our tests, the amount of winter killing was related to the previous year's treatment and to the degree of blackspot control obtained (Tables 3, 4, 5). In 1955 and 1956, the greatest amount of winter killing of Golden Masterpiece rose plants was found in plots with the highest blackspot rating the previous year (Table 3). Untreated Golden Masterpiece roses were injured more severely than untreated Red Radiance, particularly in the spring of 1955, but Golden Masterpiece treated with sulfur plus ferbam, sulfur plus copper, or zineb had less winter killing than did Red Radiance treated with the same materials. These differences were still evident, but they were not so striking in 1956. This finding indicates that Golden Masterpiece roses would be more resistant to winter injury than would Red Radiance roses if blackspot were not a factor. Winter killing in 1956 in the captan-treated plots was down to the level of that in the plots that had received the better fungicidal treatments, even though blackspot control with captan was only moderately effective in 1955. Of interest is the smaller amount of winter killing in 1956 than in 1955 when copper alone was used. Also, there was less blackspot on plants in these plots, as indicated by blackspot ratings made June 27, 1955. In contrast, blackspot control was poor in the copper-treated plots in 1954, and winter killing was severe in these plots in the spring of 1955. A possible explanation for the poor control of blackspot in 1954 is that 65 per cent sulfur dust was substituted for the copper dust in September, 1954, to avoid copper phytotoxicity during the cool fall weather. This substitution was not made in September, 1955.

There appeared to be some relation between mite control and winter killing (Table 4), but because of the variability in spider mite counts and in winter injury this relation could not be established statistically. Best mite control was obtained with Aramite and least with ovex. The greatest amount of winter killing occurred in plots where neither a fungicide or a miticide was used. Least winter killing occurred in plots where control of both blackspot and mites was good.

TABLE 4.—*Winter killing of 5 varieties of roses treated with various combinations of fungicides and miticides*

Fungicidal treatment (1954)	Percentage of wood killed (March, 1955) in plots treated in 1954 with indicated miticide [a]				
	None	Aramite	Ovex	Malathion	Average
None	86.8	70.7	74.0	72.1	75.8
Karathane	87.1	67.8	69.5	70.8	73.8
Copper	72.5	62.6	74.5	67.8	69.4
Captan	53.6	46.0	51.3	61.3	53.1
Sulfur-ferbam	45.0	34.3	34.3	36.0	37.4
Sulfur-copper	41.6	34.0	31.3	42.6	37.4
Average	64.4	52.6	55.8	58.4	

[a] Each value based on 4 replicates and 5 varieties.

TABLE 5.—*Number of live canes remaining in April, 1955, after pruning out winter-injured canes, of Red Radiance and Golden Masterpiece roses given different fungicide and miticide treatments in 1954*

Treatment received during the 1954 growing season	Average number of live canes per plot	
	Red Radiance	Golden Masterpiece [a]
Fungicides		
None	0.5	0.1
Karathane	0.3	0.1
Copper	0.4	0.8
Captan	1.8	4.6
Sulfur-ferbam	1.2	8.4
Sulfur-copper	3.2	7.7
LSD at odds of 19:1	1.4	1.8
Miticides		
None	1.0	2.9
Ovex	2.0	3.7
Malathion	1.3	3.2
Aramite	3.0	4.7
LSD at odds of 19:1	1.2	

[a] The differences between miticides are not significant by the *F* test.

On March 26, 1955, after winter-injury ratings were made, a very severe late-season freeze occurred. Although all plants had been pruned prior to this freeze, it became necessary to reprune. The average numbers of good canes remaining are given in Table 5. Again, more canes survived in plots treated previously with an effective fungicide than in those where control was not obtained, and differences were greatest in the variety Golden Masterpiece. More canes survived in Aramite-treated plots than in those where a miticide was not used. Many plants failed to survive this late freeze, and counts of dead plants June 17 revealed that only 1 of 480 Golden Masterpiece plants and 3 of Red Radiance died, whereas over 10 per cent of the Chief Seattle plants and 4.6 per cent of the Peace and Christopher Stone plants failed to survive.

DISCUSSION.—McClellan (5) stated that "blackspot remains the outstanding disease of roses and, because of the defoliation it produces, it is apparently indirectly responsible for much of the winter injury, canker, dieback and general lack of vigor in roses." Lyle (3) also reported that the loss of foliage due to blackspot resulted in weakened bushes, dieback, low-grade bushes for market, and poor growth or survival of the plants following transplanting. He also stated (4) that defoliation by blackspot reduces flower production and increases susceptibility to winter killing and that severe infection decreases cane size of bushes, diminishes number and size of blooms, lightens flower color, and decreases fragrance. Carrier (1) reported that complete defoliation of Frau Karl Druschki roses decreased their hardiness and that defoliation in August decreased hardiness more than did defoliation in September. Defoliation by the blackspot fungus also lowers the vigor of the plant and may predispose it to attacks by other fungi. Ratzek (9) demonstrated that removing leaves by hand induces dieback, which presumably is due to Diplodia. Lyle and Massey (2) concluded that the Diplodia type of dieback is not a specific disease of roses but is a secondary development resulting from factors, such as blackspot, that lower the vigor of the plant.

Our experimental results demonstrate that blackspot results in lowered vigor and production of roses and that the reduced vigor makes them susceptible to winter injury. Differences in flower yield and in winter killing due to differences in blackspot control were less for the variety Red Radiance, which was not readily defoliated by blackspot infection, than for the variety Golden Masterpiece, which was severely defoliated by blackspot. Spider mites also reduce vigor of mite-susceptible varieties and thus make them more vulnerable to winter injury. Canker development follows winter injury closely.

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LITERATURE CITED

1. CARRIER, L. E. 1953. Environment and rose hardiness. Proc. Am. Soc. Hort. Sci. **61:** 573-580.
2. LYLE, E. W., AND L. M. MASSEY. 1941. Die-back of roses. Am. Rose Ann. **26:** 177-179.
3. LYLE, E. W. 1944. Control of blackspot of roses with sulfur-copper dust. Texas Agr. Expt. Sta. Bull. 648, 27 p.
4. LYLE, E. W. 1956. Development and production of pathogen free propagation material of ornamental plants. X. Development of rose propagative material free of blackspot. Plant Disease Reptr. Suppl. 238: 91-92.
5. MCCLELLAN, W. D. 1953. Survey of rose diseases 1953. Am. Rose Ann. **38:** 70-81.
6. MCCLELLAN, W. D., F. F. SMITH, AND E. A. TAYLOR. 1955. Control of blackspot of roses with dust combinations containing fungicides, insecticides and miticides. (Abs.) Phytopathology: **45:** 465.
7. MCCLELLAN, W. D., F. F. SMITH, AND E. A. TAYLOR. 1955. Combination dusts for pests and diseases of roses. I-IV. Am. Rose Mag. **13** (2): 4-6; (3) 8-9; (4) 6, 7, 24; (5) 8, 27. (The 3 authors are in different order in each of the 4 parts.)
8. MCCLELLAN, W. D., E. A. TAYLOR, AND F. F. SMITH. 1956. Effect of fungicides in miticide-fungicide dust combinations on winter carryover of the fungus causing blackspot of roses. (Abs.) Phytopathology **46:** 20.
9. RATSEK, J. C. 1937. The probable effect of peduncle abscission on the incidence of "die-back" of roses. Proc. Am. Soc. Hort. Sci. **35:** 788-794.
10. SMITH, F. F., E. L. GOODEN, AND E. A. TAYLOR. 1955. Effect of diluents on the acaricidal action of malathion and Aramite in dusts. J. Econ. Entomol. **48:** 762.
11. TAYLOR, E. A., W. D. MCCLELLAN, AND F. F. SMITH. 1956. 1955 results with fungicide-miticide combinations on roses. Am. Rose Mag. **13** (17): 4-5, 23, 25-27.

EFFECTS OF X RAYS ON THE GERMINATION OF CONIDIA OF THE OAK WILT FUNGUS [1]

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SUMMARY

Dosages of medium-hard X rays that inhibited secondary spore formation by conidia of the oak wilt fungus *Endoconidiophora fagacearum* Bretz did not affect the formation of germ tubes. At certain higher dosages, abortive knob-like germ tubes developed from conidia and then ceased growing. This phenomenon indicates a high degree of interference with growth. As X-ray dosages increased, the number of conidia that germinated decreased and the time interval prior to germination and secondary spore formation increased.

Considerable research has been expended in the study of the effects of ionizing radiations upon biological systems. As far back as 1917, Levin and Levine (3) conducted tests which indicated that ionizing radiations might play a part in the therapy of plant diseases. Work by Rivera (4) in 1926 confirmed these results. Waggoner and Dimond (8), Anderson and Hart (1), and others have reported on attempts at plant disease therapy by ionizing radiations. This paper deals with 1 facet of a research into possible effects of ionizing radiations upon the host-parasite relationship in oak wilt, specifically, the effects of medium-hard X rays upon the germination of the spores of the oak wilt fungus, *Endoconidiophora fagacearum* Bretz, *in vitro*.

LITERATURE REVIEW.—X-radiation had little effect upon the germination of ascospores of Chaetomium in experiments reported by Dickson (2); however, he noted that when single germinated spores were irradiated, some grew for a short time and then suddenly ceased development. Uber and Goddard (7) reported similar results when ungerminated spores of Neurospora were irradiated. They also noted that the time interval prior to germination of the spores increased as dosage was increased. Schmitt (6) observed the same phenomenon when sporidia of *Ustilago zeae* were subjected to irradiation by monochromatic ultraviolet light. Rodenhiser and Maxwell (5), reporting on the effects of X-radiation on the germination of *Ustilago hordei*, stated that as much as 80 per cent germination was obtained from chlamydospores irradiated at 300 kr (kiloroentgens), but development ceased after formation of the promycelium.

MATERIALS AND METHODS.—Cultures derived from a single spore of *E. fagacearum* were used in these studies. Prior to irradiation, all cultures were kept on potato-dextrose agar at 23°C for 5–7 days. A spore suspension was made in sterile distilled water, and droplets of this suspension were aseptically transferred, several hours prior to irradiation, to standard petri plates that contained 10 ml of water agar. The suspensions were adjusted to a concentration such that 15–25 spores could be viewed in 1 field under 430 diameters magnification. Previous tests indicated that this concentration of spores would allow for good germination. The area that contained the spores was defined by drawing a circle on the underside of each plate with a marking pencil. During irradiation, a section of Masonite Prestwood, ⅛ in. thickness, was placed over the culture. Despite periods of up to 60 minutes within the X-ray machine, contamination of the plates was negligible.

Spore suspensions were placed within a circular area of ½-in. diameter in order to preclude significant error due to variation in dosage received between the outermost spores and those located centrally in the droplet. The droplets were dispersed to form a thin film over the agar, so that all spores were considered as being in 1 plane. Cultures were irradiated singly so that each culture occupied, in turn, the same spot on the platform within the X-ray unit as the other spore suspensions. Each spore suspension was in approximately the same plane in relation to the X-ray beam, since each plate contained the same quantity of media.

A Hayes X-ray Unit, Model 70, was used as the source for the X rays. The design of the cooling system of this unit permitted continuous irradiation. Dosimetry readings were calculated by use of a Victoreen Ionization Chamber. Determinations were made 46 mm from the tube, with the Prestwood interposed between the tube and the dosimeter, this being the distance from the tube at which all suspensions were maintained during irradiation. Dosimetry readings indicated that spores received 6 kr(kiloroentgens)/minute. This figure was considered to have a validity of ± 10 per cent. A tube current of 25 milliamperes and a tube voltage of 180 kilovolts were maintained throughout irradiation.

Control plates and petri plates that contained irradiated spores were incubated at 23°C during the

[1] Accepted for publication January 21, 1957.

A portion of a thesis presented to the Graduate Division, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author gratefully acknowledges counsel and guidance given him during the course of this study by Dr. W. M. Bever, University of Illinois, Dr. J. C. Carter, and the late Dr. L. R. Tehon, Illinois State Natural History Survey, Urbana, and Dr. H. Quastler, Brookhaven National Laboratory, Upton, New York.

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period of observation of irradiation effects. Counts of 200 spores on plates representing each irradiation level were made daily for 9 consecutive days following irradiation. At lower dosage levels and in the controls, where normal or near-normal germination occurred, the formation of numerous secondary spores necessitated discontinuation of counting 2 days after irradiation. The term secondary spores, in this instance, refers to spores formed from a 1- or 2-celled mycelium that normally follows germination of conidia of *E. fagacearum*. These secondary spores were formed within 36 hours following germination of the untreated conidium under the conditions of this experiment.

An exploratory run was made in the Hayes X-ray Unit, in which dosages up to 500 kr were attained. On the basis of the results obtained in this run, the LD_{50} and the maximum sublethal dosage of X rays to the conidia were approximated. Three additional runs were made, the first two each representing 11 dosages from 0 to 200 kr and from 0 to 190 kr, respectively. The results of the third run, representing 36 dosage levels from 0 to 200 kr, approximated those of the preceding tests; therefore only the results of the third run are reported.

TABLE 1.—*Germination of conidia of Endoconidiophora fagacearum after exposure to various levels of X-radiation*

Dosage levels (kr)	Percentage germination at the end of indicated time periods (in hours) after irradiation [a]				
	36	60	84	99	120
0	70.5	89.0	—	—	—
5	68.0	83.0	—	—	—
10	60.0	84.5	—	—	—
15	65.0	86.5	—	—	—
20	30.0	66.5	—	—	—
25	12.0	12.0	13.0	—	—
30	11.5	12.5	13.0	—	—
35	8.0	9.5	9.0	10.5	—
40	4.5	6.0	8.5	12.0	17.0
45	4.0	6.0	7.0	10.5	12.0
50	6.0	6.5	6.5	12.0	11.5
55	4.0	4.0	5.5	7.0	9.0
60	3.5	3.5	4.0	4.0	4.5
65	1.0	3.0	3.0	3.0	4.0
70	2.5	2.0	3.0	3.0	4.0
75	1.5	2.0	2.5	2.0	2.0
80	4.0	4.0	3.5	4.0	4.5
85	2.0	2.5	2.0	1.5	2.0
90	0.5	1.0	2.0	2.5	2.0
95	0.0	0.0	0.0	0.5	1.0
100	0.0	0.0	1.0	1.0	1.0
105	0.0	0.0	0.0	0.5	0.0
110	0.0	0.0	0.0	0.5	1.0
115	0.0	0.0	0.0	0.0	0.0
120	0.0	0.0	0.0	0.0	0.5
125	0.0	0.0	0.0	0.0	0.0
130	0.0	0.0	0.0	0.0	0.5
135	0.0	0.0	0.0	0.0	0.0
140	0.0	0.0	0.0	0.5	0.5
145	0.0	0.0	0.0	0.0	0.0
150	0.0	0.0	0.0	0.0	0.0
160	0.0	0.0	0.0	0.0	0.0
170	0.0	0.0	0.0	0.0	0.0
180	0.0	0.0	0.0	0.0	0.0
190	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	0.0	0.0	0.0

[a] A total of 200 spores counted at each dosage level at each time period. Where a dash occurs, secondary spores were numerous, thereby making counts impossible.

RESULTS.—Germination of conidia of the oak wilt fungus after exposure to X rays is recorded in Table 1. Secondary spores were produced by germinated conidia after having been exposed to dosages up to 50 kr. At the 50-kr dosage level, 13 spores of the 200 counted had germinated at 84 hours following irradiation; however, only 1 spore produced secondary conidia. After exposure to 40 kr, 2 of the 12 spores that had germinated at 60 hours produced secondary conidia, and 8 of the 17 spores that had germinated at 84 hours produced secondary conidia. The percentage of germinated spores that produced secondary conidia decreased sharply at dosages above 20 kr (Fig. 1,A). At dosages of 20 kr and less, secondary spores were produced by 36 hours (Fig. 2,A). At dosages above 20 kr, the higher the dosage, the longer

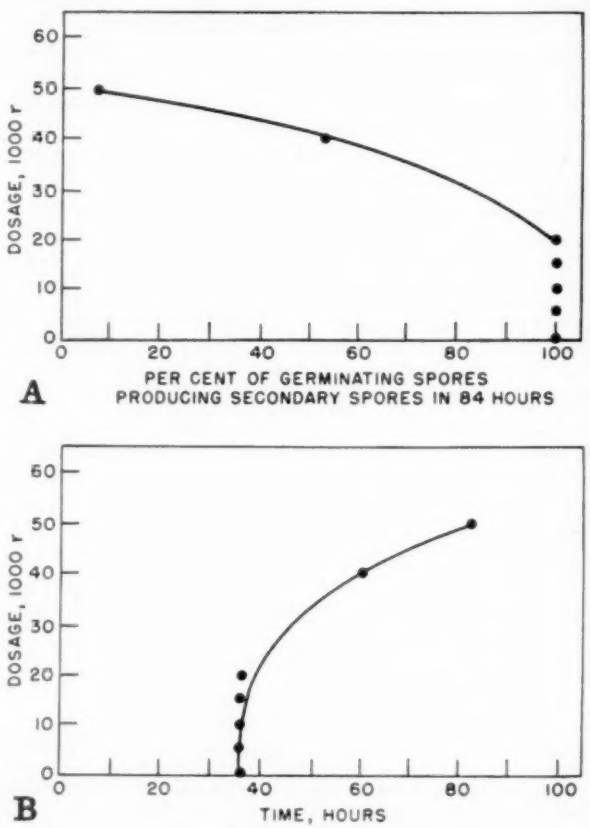


FIG. 1. A) Effect of X rays on secondary spore formation by conidia of *Endoconidiophora fagacearum*. Relation between percentage of spore germinations giving rise in 84 hours to secondary spores and X-ray dosage. Number of untreated spores germinated at 84 hours is considered 100 per cent. B) Beginning of secondary spore production in relation to time and X-ray dosage.

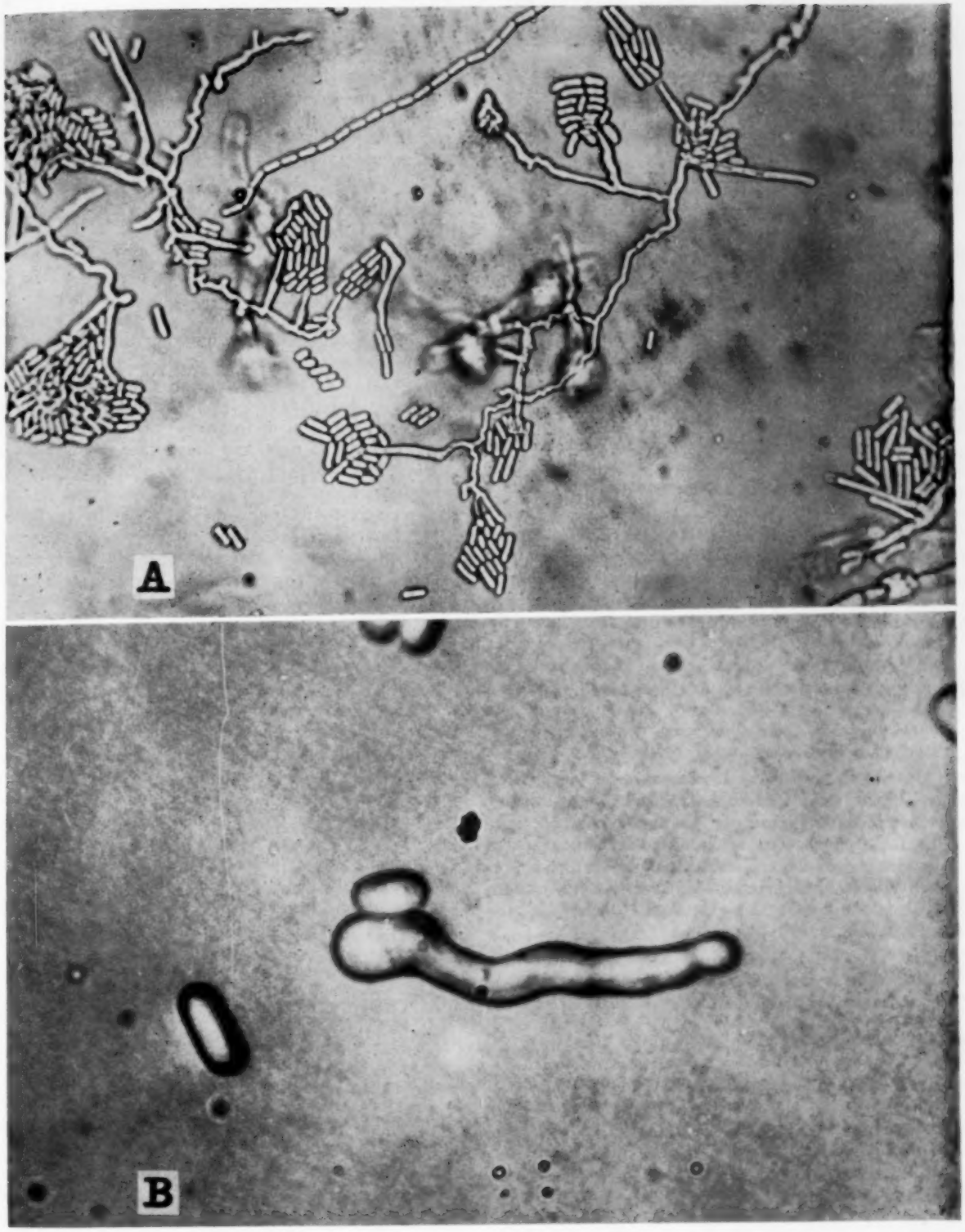


FIG. 2. A) Germinated conidia in a control culture of *Endoconidiophora fagacearum* after 36 hours of incubation. Production of secondary spores is prolific (approximately ×650). B) Conidium, photographed 8 days following irradiation at the 60-kr dosage level, exhibiting an abortive, sterile germ tube (approximately ×2500).

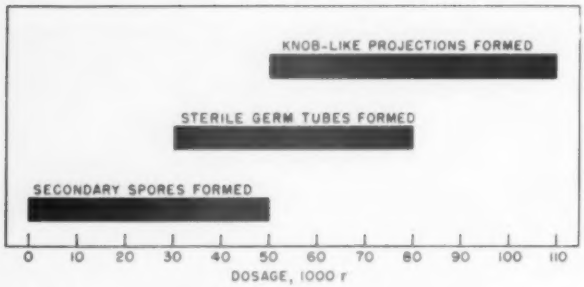

KNOB-LIKE PROJECTIONS FORMED
STERILE GERM TUBES FORMED
SECONDARY SPORES FORMED
0 10 20 30 40 50 60 70 80 90 100 110
DOSAGE, 1000 r

FIG. 3. Ranges of X-ray dosages within which germinating spores of *Endoconidiophora fagacearum* produced secondary spores, sterile germ tubes, and knob-like projections from the spores.

was the delay in the inception of germination and in secondary spore formation (Fig. 1.B).

At dosages of 30–80 kr, many conidia formed abortive germ tubes. These germ tubes were sometimes 2–30 times the lengths of the spores but more commonly were 2–6 times the lengths of the spores (Fig. 2,B). After a short period of elongation, these germ tubes stopped developing and did not produce secondary conidia.

At dosage levels of 50–110 kr, many spores formed short knob-like projections and then ceased development. The knob-like formations frequently were accompanied by a swelling of 1 end of the spore. The formation of these knobs became less frequent as the dosage increased to 110 kr, when the predominating effect was total lethality; however, knobs formed on 6 spores exposed to X-ray dosages as high as 140 kr. These conidia were examined at intervals up to 216 hours following irradiation, and it was noted that they had not resumed growth at that time.

The relation of the formation of secondary spores, sterile germ tubes, and knob-like projections to dosage is illustrated in Figure 3. This figure does not indicate the frequency at which these formations occur but depicts the range throughout which each was observed (the occurrence of knob-like projections on 6 conidia at dosages higher than 110 kr is not indicated in this figure).

The LD_{50}, if interpreted as the dosage at which 50 per cent of the conidia had not germinated at 36 hours, ranged from approximately 16 to 17 kr in the 3 tests. If the LD_{50} were interpreted as the dosage at which 50 per cent of the spores failed to germinate at 120 hours, the range would be 18 to 19 kr. Spores that had not germinated by 120 hours usually failed to germinate subsequently.

Slight fluctuations in spore germination figures at some time levels in Table 1 were attributed to the counting of different spores at each time level. For example, at the 70-kr dosage level, 2.5 per cent of the spores were recorded as having germinated at the 36-hour time level and 2.0 per cent as having germinated at the 60-hour time level.

DISCUSSION.—Untreated conidia of *E. fagacearum* germinated and formed secondary conidia within 36 hours under the conditions of the experiment. As X-ray dosages increased, the time interval prior to germination and secondary spore production increased and the number of conidia that germinated decreased. These results agree with those of other workers (2,6, 7). At still higher dosages, the growth processes underwent a period of inactivity, then functioned for a short period of time, and then suffered a relapse.

Uber and Goddard (7), after observing abortive germ-tube formation of the type described in this paper, suggested that this growth might be attributed to a chemical response and might not be regarded as growth in the ordinary sense. In the experiments reported in this paper, abortive germ tubes formed from irradiated conidia of *E. fagacearum* were devoid of cytoplasmic content. This observation would not sustain the conclusion that the cytoplasm had not increased in quantity as the germ tube elongated, but it was noted that the germ tubes that emanated from untreated conidia contained cytoplasm.

Rodenhiser and Maxwell (5) observed this phenomenon and proposed that it be called "delayed killing." This term presupposes that the spores were living when germination occurred, but died following germination under the influence of a delayed lethal factor.

It is hoped that future studies of the biochemical changes that take place within a conidium under the impetus of ionizing stimuli will resolve the problem of this "ephemeral growth" phenomenon.

SECTION OF APPLIED BOTANY AND PLANT PATHOLOGY
ILLINOIS STATE NATURAL HISTORY SURVEY
URBANA, ILLINOIS

LITERATURE CITED

1. ANDERSON, R. V., AND HELEN HART. 1956. Effects of ionizing radiations on the host-parasite relationship of stem rust of wheat. (Abs.) Phytopathology **46:** 6.
2. DICKSON, H. 1932. The effects of x-rays, ultraviolet light and heat in producing saltants in Chaetomium cochliodes and other fungi. Ann. Botany **46:** 389-405.
3. LEVIN, I., AND M. LEVINE. 1917. The influence of X-rays on the development of crown gall. Proc. Soc. Exptl. Biol. Med. **15:** 24-25.
4. RIVERA, V. 1926. Azione dei raggi × sopra i tumori vegetali. Riv. Biol. (Perugia) **7:** 449-465.
5. RODENHISER, H. A., AND L. R. MAXWELL. 1941. Effect of X-radiation on the germination of chlamydospores of Ustilago hordei. Phytopathology **31:** 175-181.
6. SCHMITT, C. G. 1940. Cultural and genetic studies on Ustilago zeae. Phytopathology **30:** 381-390.
7. UBER, F. M., AND D. R. GODDARD. 1934. Influence of death criteria on the X-ray survival curves of the fungus, Neurospora. J. Gen. Physiol. **17:** 577-590.
8. WAGGONER, P. E., AND A. E. DIMOND. 1952. Examination of the possibility of therapy of plant disease with ionizing radiation. Phytopathology **42:** 599-602.

EFFECTS OF GENETIC SEGREGATION IN PHASEOLUS VULGARIS ON THE SYMPTOMS INDUCED BY BEAN YELLOW MOSAIC VIRUS [1]

James R. Baggett [2]

SUMMARY

A genetically variable population of bean plants inoculated with a pod-distorting strain of bean yellow mosaic virus showed a wide range of symptom expressions. Symptoms expressed by F_3 progeny types from a cross between resistant Great Northern U. I. 31 and susceptible O.S.C. 21 (Blue Lake) included those of each parent, as well as a wide range of extreme and intermediate types differing in form as well as severity.

Attempts to classify virus strains often stress symptom expression in standard plant materials. It is evident, however, that the individual plant genotype is important in the host-virus interaction. Variability within plant groups may thus affect the results of host-range studies and create confusion in the field of virus research. This paper demonstrates that the symptoms induced by a single strain of bean yellow mosaic virus (BYMV) may differ widely because of genetic segregation resulting from hybridization of 2 varieties within the same plant species.

Since 1952, studies have been made at Oregon State College on the inheritance of resistance to BYMV in the garden bean, *Phaseolus vulgaris* L. When a population of F_3 families from a cross of Great Northern U.I. 31 × O.S.C. 21 (Blue Lake) was inoculated with a single pod-distorting strain of this virus, there was a wide range of symptom form and severity in a uniform environment. Many groups of F_3 families were inoculated, but the present report deals only with a single group tested at 1 time under the same greenhouse conditions. The results of the corresponding inheritance studies will be published elsewhere.

MATERIALS AND METHODS.—*The virus strain.*—The strain of BYMV used was isolated from Blue Lake beans in the Williamette Valley of Oregon and designated as the Y strain. In addition to the usual mottle, stunting, necrosis, and epinasty, this strain characteristically causes pod distortion in susceptible varieties such as Blue Lake. All tests for seed transmission have been negative. Bean common mosaic virus established in F. M. 65 Blue Lake beans completely protected against the Y strain. Thermal inactivation *in vitro* occurs at about 60°C.

The Y strain has been tested on more than 100 varieties of beans. Of these, Great Northern U.I. 16 and U.I. 31 have shown strong resistance, and possible degrees of resistance have been observed in Puregold, High Bush Logan, U.S.D.A. B-1387 (Vegetable Breeding Lab., Charleston, S.C.), and several foreign varieties.

In limited tests on species other than *Phaseolus vulgaris*, this strain has been transferred to *Vicia faba* L., *Trifolium incarnatum* L., *T. hybridum* L., *Melilotus officinalis* (L.) Lam., and *Pisum sativum* L. (var. Thomas Laxton, Freezonian, and Rocket). No infections were observed in *Glycine max* (L.) M., *Vicia villosa* Roth., *Trifolium pratense* L., *T. subterraneum* L., *T. repens* L., *Melilotus alba* Desr., or *Pisum sativum* L. (var. W. R. Perfection, Alaska, Little Marvel, and Surprise).

Bean varieties and definition of resistance.—The Great Northern U.I. 31 field bean, developed in Idaho for resistance to curly top and bean common mosaic viruses,[3] was used as the resistant parent. When inoculated with the Y strain, this variety developed necrotic local lesions on the inoculated leaves, followed by spreading vein necrosis that often eventually covered most of the blade (Fig. 1). No other symptoms occurred when the plants were inoculated by rubbing in the presence of abrasive. When young plants were approach-grafted to systemically infected plants of another variety, a small percentage developed several patches of vein necrosis in the upper leaves, from which the virus was recovered. A resistant variety is thus defined as one in which no systemic symptoms occur when inoculated by rubbing.

The susceptible parent, O.S.C. 21, a uniform-breeding line selected from the F.M.-1 strain of Blue Lake, developed a severe systemic infection when inoculated by rubbing (Fig. 3). Necrosis of inoculated leaves was slight or absent.

Procedures.—Seeds of 267 F_3 families were planted in greenhouse groundbeds on March 28, 1955. Two randomly distributed plots of 5 plants each were grown for each family, whereas each parent was grown in 4 randomly distributed plots. Plants in the early primary-leaf stage were dusted lightly with 400-mesh silicon carbide powder and rubbed with a 1:5 dilution of juice from young infected leaves.

The weather was intermittently cloudy and sunny during the test period. Night temperatures ranged from 60° to 65°F, daytime temperatures from 70° to 85°F.

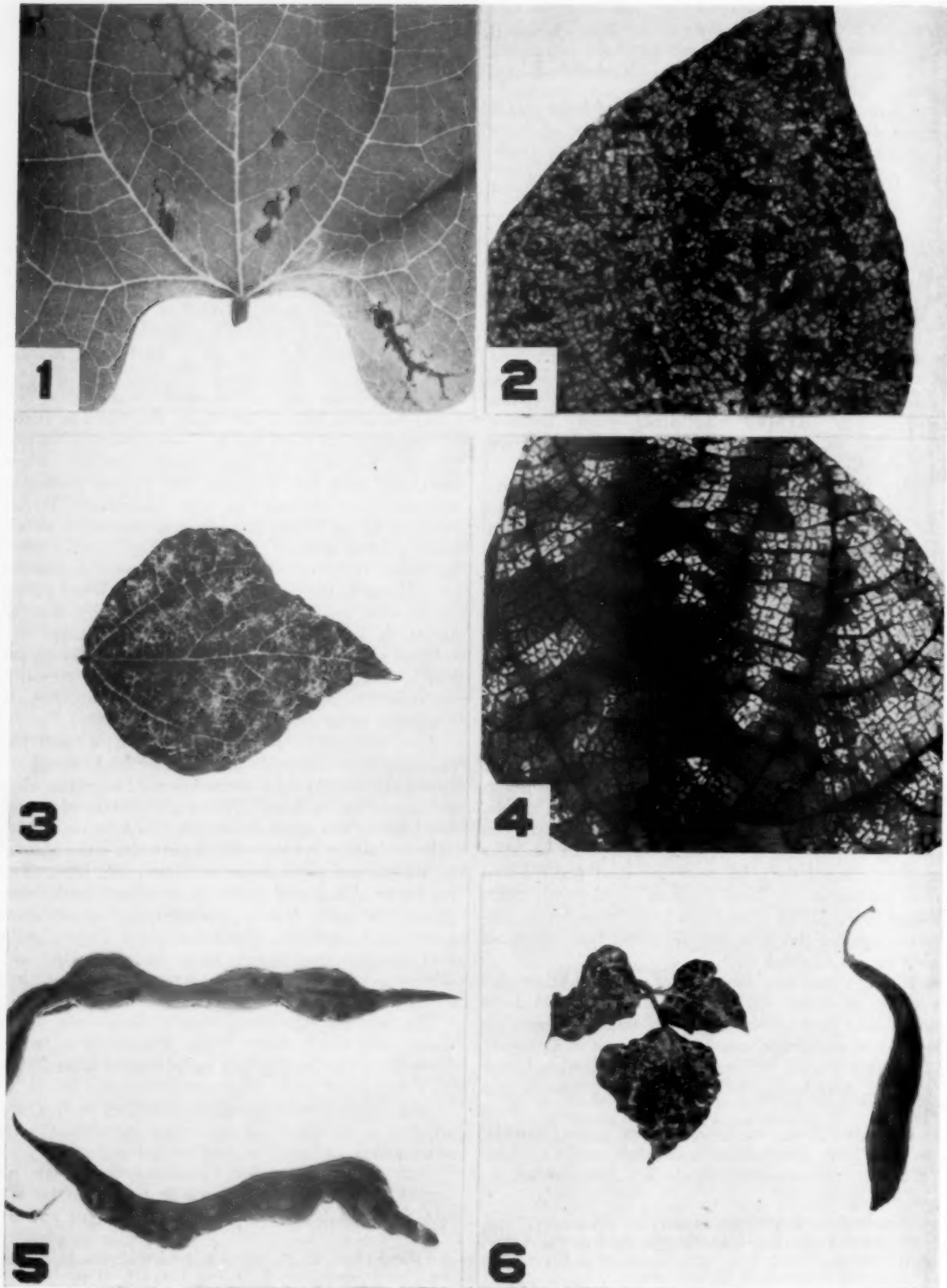
The families were classified according to type and severity of symptoms 75 days after inoculation, when most plants had pods of medium maturity.

RESULTS.—The various symptoms that were observed alone or in combination in the tested families are classified below according to their type and the

[1] Accepted for publication January 23, 1957.
Technical Paper No. 1012, Oregon Agricultural Experiment Station.

[2] Research Assistant in Horticulture.

[3] Hungerford, C. W. 1952. Disease resistant field beans for Idaho. Idaho Agr. Expt. Sta. Cir. 118, 11 p.


1
2
3
4
5
6

part of the plant affected.

I. Inoculated leaf symptoms (Fig. 1)
   A. Discrete necrotic local lesions
   B. Vein necrosis—either as net necrosis spreading from discrete lesions or occurring alone in varying degrees

II. Systemic symptoms
   A. Necrotic
      1. Pod necrosis—severe red necrotic areas in combination with pod distortion (Fig. 5)
      2. Discrete necrotic lesions—often appearing as an early symptom on upper nonmottled leaves or as necrotic flecks on mottled leaves
      3. Vein necrosis—as spreading areas from discrete lesions or appearing in patches or generally over the surface of upper leaves (Fig. 4)
      4. Stem necrosis—occurring at any stage of development and ranging in severity from a chronic mild condition to death of the terminal or of the entire upper part of the plant
   B. Nonnecrotic
      1. Systemic mottle—typical mosaic symptoms, with degrees of leaf distortion and plant stunting ranging from very mild to extremely severe, with or without pod distortion (Fig. 2, 3, 6)
      2. Pod distortion—ranging from very slight to severe roughening and twisting (Fig. 5, 6)

The above symptoms appeared in many degrees and combinations and at different stages of plant development. Individual F_3 families tended strongly to produce uniform symptoms, although genetically segregating families included plants of different symptom types. For simplicity, the families were classified somewhat arbitrarily into 8 classes, according to the combination of symptoms that occurred (Table 1).

In Table 1, systemic mottles are divided into 3 categories: 1) extremely severe, 2) severe, and 3) mild. Plants with the extremely severe type (Fig. 6, 7) were strikingly dwarfed and distorted, leaf diameter was less than 1/5 of normal, and plant height was less than 1/10 of normal. Plants in the intermediate class, Class 2, were moderately to severely dwarfed and showed considerable leaf distortion. In mildly mottled plants, Class 3, distortion was slight or absent, and leaf size was normal or nearly so. Pod distortion was common in Classes 1 and 2 and present in some families of Class 3 but many severely mottled plants did not show distinct pod distortion. Plants in Classes 5 and 6 varied with respect to systemic mottles (upon which the severe necrotic reactions were superimposed).

There was no strong relationship apparent between severity of mottle and the type of symptoms on inoculated leaves, although in Class 5 through 8 there was a tendency for the production of discrete necrotic

TABLE 1.—*A classification of F_2 families of O.S.C. 21 × G.N. 31 based on type and severity of symptoms following mechanical inoculation with bean yellow mosaic virus*

Class based on severity of systemic symptoms		No. plants in class	No. plants in class with (+) and without (−) systemic necrosis	No. of plants with indicated extent or type of necrosis on inoculated leaves		
Class no.	Symptom[a] type			Slight or absent	Net necrosis	Distinct lesions
1	Extremely severe mottle	19	0 (+)			
			19 (−)	13	4	2
2	Severe mottle	144	12 (+)	10	0	2
			132 (−)	120	7	5
3	Mild mottle	61	32 (+)	29	0	3
			29 (−)	28	0	1
4	Severe and mild mottle—segregating	29	22 (+)	21	1	0
			7 (−)	7	0	0
5	Lethal systemic necrosis	6	6 (+)	3	0	3
6	Late general necrosis	6	6 (+)	3	0	3
7	Light systemic vein necrosis only	1	1 (+)	0	0	1
8	No systemic symptoms	4	4 (−)	1	0	3
	Totals	270	79 (+)	66	1	12
			191 (−)	169	11	11

[a] Mottling in Class 1 more severe than that shown by O.S.C. 21, the susceptible parent; mottling in Class 2 typical of that shown by the susceptible parent. G.N. 31, the resistant parent, produces no symptoms when mechanically inoculated.

←

FIG. 1–6. Symptoms induced by the Y strain of bean yellow mosaic virus on beans.—Fig. 1. Necrotic lesions on an inoculated leaf, characteristic of the resistant parent and many F_2 families.—Fig. 2. Mild but distinct mottle, with little distortion or effect on leaf size.—Fig. 3. Leaflet from O.S.C. 21, typical of the severe mottle type.—Fig. 4. General systemic vein necrosis and yellowed areas.—Fig. 5. Severe pod distortion and necrosis.—Fig. 6. Leaf and pod from a plant with extremely severe symptoms.

FIG. 7. Extremely severe symptoms of an F_2 plant, 60 days old and 11–12 in. in height.

lesions. Systemic necrosis apparently was related to mottle severity, being absent in Class 1, present in 10 per cent of the Class 2 families and in over 50 percent of the Class 3 and 4 families. Plants in Classes 5, 6, and 7 were so classified originally on the basis of systemic necrosis. The amount of necrosis increased progressively from Class 1 to Class 6, whereas only slight systemic necrosis without mottling was shown by plants in Class 7. Plants in Class 8 were resistant because of complete localization.

DISCUSSION.—The foregoing observations demonstrate the strong effects of genetic segregation on the reaction to infection by bean yellow mosaic virus. From 2 parents, each producing a uniform reaction in a particular environment, it was possible to derive a population of genotyes that varied greatly in their reaction to infection. Virtually every type of symptom described for the reported strains of BYMV[4,5,6,7] in a large number of bean varieties has been observed in this segregating population. Actually, the range of symptoms produced in this test by a single virus strain extends beyond the limit usually accepted for the classification of BYMV strains in bean varieties. Although the genetic studies (Table 1) indicate that 3 major genes may control differences between susceptible and resistant plants, it is probable that the variation in degree and form of symptoms in susceptible types is influenced by many modifying genes. The diversity of systemic necrotic symptoms probably is conditioned by combinations of factors for systemic infection from the susceptible parent with factors for the localized necrotic reaction of G. N. 31.

This study was made in a uniform environment—a situation favorable for the comparison of different genetic materials. That environment may affect symptom expressions is well known. For example, the distinct local lesions usually produced on inoculated leaves of G.N. 31 are absent or indistinct under winter conditions in this locality. Furthermore, the terminal of O.S.C. 21 often is killed at an early stage during the winter by the same virus strain that causes only a severe mottle in spring and summer. The genetic effects discussed are meaningful only in terms of a particular environment and a single virus strain. Other strains may produce an entirely different symptom picture in such a breeding population.

Any study in which virus symptom expression is stressed should involve some consideration of the inherited potentialities of the plant materials, in addition to the well-known differences among virus strains and the effects of environment, since each of these factors may influence the form as well as the severity of disease expression.

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[4] Grogan, R. G., and J. C. Walker. 1948. A pod-distorting strain of the yellow mosaic virus of bean. J. Agr. Research 77: 301-314.

[5] Pierce, W. H. 1934. Viroses of the bean. Phytopathology 24: 87-115.

[6] Thomas, H. R., and W. J. Zaumeyer. 1953. A strain of yellow bean mosaic virus producing local lesions on tobacco. Phytopathology 43: 11-15.

[7] Zaumeyer, W. J., and H. H. Fisher. 1953. A new necrotic-lesion producing strain of yellow bean mosaic. Phytopathology 43: 45-49.

THE ISOLATION OF THE TOXIN OF HELMINTHOSPORIUM VICTORIAE [1]

Ross B. Pringle and Armin C. Braun

SUMMARY

The soluble metabolite responsible for the toxic manifestations of the Helminthosporium blight disease of oats has been isolated. The purified toxin shows the same host specificity as does the causal fungus and produces symptoms in susceptible oat plants that are indistinguishable from those produced by it. The toxic substance was isolated by growing the fungus in shallow layers in modified Fries' No. 3 basal medium at 25° C for 28 days. After removal of the fungal mycelium, the filtrate was acidified and concentrated *in vacuo* to one-tenth its original volume. Inert material was removed from the concentrate by precipitation with methanol. After further evaporation *in vacuo* to remove methanol, the toxin was extracted into butanol and purified by chromatography on columns of alumina and starch. The purity of the toxin was checked by further chromatography on a variety of adsorbents, by paper chromatography, by ionophoresis, by counter-current distribution, and by the preparation of derivatives. The isolated toxin inhibits the growth of susceptible oat roots at a concentration of 0.01 μg per ml. It is thus one of the most potent toxins known. The biologically active material appears to be polypeptide in nature.

The Helminthosporium blight of oats was first recognized in the field in April, 1945 (5). Yielding ability of Victoria-Richland derivative oats was reduced as much as 50 per cent by this blight in certain oat-growing regions of the Midwest. Losses were even greater in 1946 and 1947. These Victoria-Richland derivatives had shown great promise because of their resistance to the crown rust fungus, especially to those races for which there had previously been no source of resistance.

The causal fungus was first recognized as a new species in 1946 by Meehan and Murphy, who named it *Helminthosporium victoriae* (6). It was found to remain localized in the tissues of the host but to produce an extracellular toxin that diffused through the plant, causing a bronzing followed by chlorosis and necrosis (7). When the fungus was grown on a synthetic medium, the toxin appeared in the culture filtrate and was capable of inducing symptoms similar to those caused by the pathogen on its host (3). Luke and Wheeler developed a medium in which high yields of the toxin were produced (4).[2] Their crude culture filtrates killed seedlings of sensitive varieties of oats at dilutions of 1:1000; root growth of seedlings treated with dilutions of 1:1,000,000 was only about half that of untreated seedlings. These authors reported that they were able to concentrate the toxic material by adsorption on charcoal and elution with acetone.

This toxin is of particular interest because of its highly selective action. Its isolation was undertaken in order to obtain pure material for biochemical studies on its mechanism of action. The isolation proved to be exceedingly difficult because upon purification the toxin became extremely unstable. It was even more unstable than the toxin produced by *Pseudomonas tabaci* (Wolf & Foster) Stevens (10).

The toxin was concentrated sufficiently to inhibit growth of roots of susceptible seedlings when it was diluted to 0.01 μg per ml. The isolated material was shown to be essentially homogeneous.

Although we did not succeed in eluting the toxin from several kinds of charcoal with acetone, as was reported by Luke and Wheeler, we nevertheless believe it to be the same toxin that they studied.

BIOASSAY.—As a guide in following the isolation of the toxin, the bioassay described below was developed. This gave rapid, reproducible, quantitative measures of toxin concentration. Seeds of a susceptible oat variety (Park C.I. 6611) were soaked in distilled water for 1 hour and the hulls gently removed by forceps.[3] The hulled seeds were allowed to germinate for 24 hours between sheets of moist filter paper. The potency of toxin concentrates could be assayed by measuring inhibition of root growth when these susceptible germinated seeds were placed in a serial dilution of the toxin in White's nutrient salts (9) buffered at pH 5.0 with McIlvaine's standard buffer. Five ml of each dilution was placed in 60 × 15-mm petri dishes, and 5 seeds were introduced into each dish. The assay was incubated 48 hours at 25° C. It was then read by taking as the endpoint the highest dilution that limited root growth to a length of 1.0 cm or less. Control roots grew to a length of 5.0 or 6.0 cm under these conditions. This procedure was found to be more reproducible than that used by Luke and Wheeler (4).

When seeds from which the hulls were removed prior to germination were placed in White's salts solution, the rate of growth of the roots was more uniform than when unhulled oats were grown in dis-

[1] Accepted for publication February 1, 1957.

The authors wish to express sincere appreciation to Miss Barbara A. Rasnick for technical assistance in producing and assaying this material.

[2] The authors are greatly indebted to Dr. H. E. Wheeler, Louisiana State University, Baton Rouge, Louisiana, for making available to them the composition of a culture medium favorable for toxin production as well as for the high-toxin-producing strain of *Helminthosporium victoriae* that was used in these studies.

[3] The authors wish to thank Dr. Frank C. Petr and Dr. Howard Rhoads of the Agronomy and Soils Department, Montana State College, Bozeman, Montana, for supplying the Park oats (C.I. 6611) and Dr. Hickman C. Murphy, Iowa State College, Ames, Iowa, for supplying Victoria oat seed.

tilled water. Buffering the solution ensured that all samples were tested at the same pH. This assay measures essentially complete inhibition of root growth. It was found very difficult to determine either directly or by interpolation the point at which root growth was 50 per cent of normal because large changes in concentration around this point caused relatively small differences in the length of roots produced.

PRODUCTION OF TOXIC FILTRATES.—In the initial studies, some 34 different strains of *H. victoriae* were grown on a variety of culture media and under various cultural conditions.[4] Most strains produced little toxin, and those that did quickly lost this ability. Two isolates derived from single spores of strain C of Luke and Wheeler were very effective producers of toxin, and they retained this ability for more than a year. These 2 isolates were used for the production of toxin. The following procedure was developed by modifying that of Luke and Wheeler (4).

One hundred Erlenmeyer flasks of 1-liter capacity were charged with 100 ml of a modified Fries' No. 3 basal medium of the following composition: ammonium tartrate, 5.0 g; NH_4NO_3, 1.0 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.13 g; sucrose, 30 g; $MnSO_4 \cdot 4H_2O$, 1.0 mg; boric acid, 1.0 mg; $CuSO_4$, 0.1 mg; $ZnSO_4 \cdot 7H_2O$, 0.1 mg; $FeSO_4 \cdot XH_2O$, 20 mg; and distilled water to make 1000 ml (2). The medium in each flask was seeded as described by Luke and Wheeler (4). After incubation at 25° C for 28 days, the culture solution containing the toxin was harvested by filtration through several layers of cheesecloth. Each such batch was assayed; if the filtrate was not active at a dilution of 1:8000, it was discarded. Average batches assayed to 1:128,000. Active filtrates were acidified to pH 3.6 with 0.1 N H_2SO_4, pooled, and processed in batches of 100 liters.

ISOLATION OF THE TOXIN.—One hundred liters of culture filtrate were evaporated *in vacuo* below 45° C in a large-capacity circulating evaporator to a volume of 10 liters. An equal volume of methanol was stirred with the solution, and the mixture was placed at 5° C for 48 hours. After this time the precipitated material was removed by filtration, washed with cold 50 per cent methanol, and discarded. The filtrate was concentrated *in vacuo* below 45° to remove methanol. A precipitate appeared as the solution was concentrated to a volume of about 8000 ml; this was removed by filtration and discarded. The filtrate was extracted in 1-liter batches with an equal volume of 1-butanol. The extraction was repeated twice, causing the aqueous phase to decrease to about 25 per cent of its original volume.

The butanol extract was concentrated *in vacuo* below 45° C to about 2000 ml. As evaporation proceeded, crystals of ammonium tartrate separated; these were removed by filtration. An equal volume of methanol was added to the filtered butanol concentrate, and the solution was subjected to chromatography in 1000-ml batches on Merck reagent aluminum oxide. A 160 × 75-mm column of this material was treated with 10 per cent H_2SO_4, washed with water until the eluate reached pH 3.5, and dried with methanol. After the 1000 ml of toxin solution in butanol-methanol had passed into the column, 500 ml of methanol was passed through, followed by 50 per cent aqueous methanol. Development with 50 per cent methanol was continued until 2 colored bands had passed down the column. This usually required about 1000 ml. After the second brown band had passed from the column, the toxin was eluted with 1000 ml of 1 per cent aqueous acetic acid. The acetic acid eluates were concentrated *in vacuo* below 45° to a volume of 1000 ml. This solution was active at about 0.02 μg of dry weight per ml of solution.

Purification beyond this stage was exceedingly difficult because of the instability of the toxin. The activity could be raised to 0.01 μg per ml by chromatography on a column of starch according to the technique of Stein and Moore (8). An equivalent of 20 mg dissolved in butanol was placed on a 40 × 1.9-cm column and developed with a solvent mixture of 75 ml water and 5 ml acetic acid dissolved in sufficient butanol to make 500 ml. Progress of the chromatography was followed by assaying aliquots of eluate.

The value of maximum activity of 0.01 μg per ml was obtained by assaying and determining dry weights from aliquots of the effluent from the starch column. The material was too unstable to dry, weigh, and assay directly. After being dried *in vacuo* at 20° C, the material darkened and failed to redissolve completely in water.

Yields of toxin were in the order of 100 mg for each 100 liters of culture filtrate processed.

EVIDENCE FOR PURITY OF THE TOXIN.—The toxin is rapidly inactivated by pH above 4.0 and by aeration. Even lyophilization of solutions caused a great loss of activity. These considerations made purification difficult; however, as many different procedures as possible were employed to make certain that the preparation was relatively free of impurities. The fact that the purified material was active at 0.01 μg per ml minimizes the probability that activity could be due to an impurity.

The purified material was chromatographed on a column of powdered cellulose (Solka-Floc BW200) prepared and run as described previously (10). In this system the toxin moved as a single component, and no increase in potency was obtained.

The purified material was chromatographed in the same solvent system on a Grycksbo paper-roll column (1). Again, only a single band of activity was found, and no increase in potency on a dry-weight basis was found.

[4] Isolates of *Helminthosporium victoriae* were very kindly supplied by the following persons: Drs. William B. Allington, Robert W. Earhart, S. S. Ivanoff, Arthur Kelman, Frances M. Latterell, Curt Leben, E. S. Luttrell, Julian H. Miller, M. B. Moore, Marr D. Simons, and Harry E. Wheeler.

The purified material was chromatographed on paper by means of the following solvent systems. The R_f values of the toxin were determined by cutting the paper into small segments and assaying each.

Butanol:benzyl alcohol:water (1:1:0.288) ..... $R_f = 0.0$
Butanol:acetic acid:water (100:1:15) ..... $R_f = 0.2$
Propanol:acetic acid:water (200:3:100) ..... $R_f = 0.7$
Propanol:phosphate buffer pH 5.0 (2:1) ..... $R_f = 0.8$
Methyl ethyl ketone:propionic acid:water (15:5:6) ..... $R_f = 0.8$

In none of these systems was there evidence of more than a single active component.

To study the homogeneity of the preparation further, ionophoresis on paper was used at those pH levels where the toxin was stable, i.e. below pH 4.0. In each of the systems, glycine-HCl buffer pH 2.5, HCl-KCl pH 1.2, 2 M acetic acid, or 1.5 M formic acid, only 1 toxic component was found. The material migrated at about the same rate as did valine. Runs were made so that the material moved across about 20 cm of paper.

The purified toxin was distributed in a countercurrent system consisting of butanol and 1 per cent acetic acid solution. Because the toxin was inactivated by this procedure, only 6 transfers were made. A single peak of activity was found in this system.

Finally, toxic activity was shown to follow the compound through a series of chemical manipulations. The toxin was first precipitated from aqueous solution by picric acid. The dried picrate was then dissolved in anhydrous acetone, from which the toxin was precipitated by dry hydrogen chloride. This precipitate, after being washed thoroughly with acetone and redissolved in water, still retained the biological activity of the toxin. Since a major percentage of activity was lost during this manipulation, it was not a satisfactory procedure for preparing an analytical sample.

The toxin appears to be polypeptide in nature. Although fresh biologically active preparations did not react with ninhydrin, this reaction was noticed as the preparations lost activity. When the toxin was hydrolyzed by 6 N HCl or by 10 N NaOH, the ninhydrin reaction became progressively stronger. Completely hydrolyzed samples showed at least 7 ninhydrin-reacting substances. An attempt is being made to characterize these compounds.

Because of the instability of this toxin, it has not yet been possible to obtain a preparation suitable for melting-point determination or for elemental analysis; however, we believe that we have succeeded in preparing material that is relatively free of impurities and is suitable for experiments on the biochemical mechanism of action of this toxin.

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NEW YORK, NEW YORK

LITERATURE CITED

1. DANIELSSON, C. E. 1953. A new type of preparative chromatographic paper column. Arkiv Kemi **5**: 173-176.
2. FRIES, N. 1938. Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Symbolae Botan. Upsalienses **3** (2), 188 p.
3. LITZENBERGER, S. C. 1949. Nature of susceptibility to Helminthosporium victoriae and resistance to Puccinia coronata in Victoria oats. Phytopathology **39**: 300-318.
4. LUKE, H. H., AND H. E. WHEELER. 1955. Toxin production by Helminthosporium victoriae. Phytopathology **45**: 453-458.
5. MEEHAN, FRANCES L. 1950–1951. Helminthosporium victoriae and other graminicolous species. Iowa State Coll. J. Sci. **25**: 292-294.
6. MEEHAN, FRANCES, AND H. C. MURPHY. 1946. A new Helminthosporium blight of oats. Science **104**: 413-414.
7. MEEHAN, FRANCES, AND H. C. MURPHY. 1947. Differential phytotoxicity of metabolic by-products of Helminthosporium victoriae. Science **106**: 270-271.
8. STEIN, W. H., AND S. MOORE. 1948. Chromatography of amino acids on starch columns. Separation of phenylalanine, leucine, isoleucine, methionine, tyrosine, and valine. J. Biol. Chem. **176**: 337-365.
9. WHITE, P. R. 1943. A handbook of plant tissue culture. Jaques Cattell Press, Lancaster, Pa. 277 p.
10. WOOLLEY, D. W., R. B. PRINGLE, AND A. C. BRAUN. 1952. Isolation of the phytopathogenic toxin of Pseudomonas tabaci, an antagonist of methionine. J. Biol. Chem. **197**: 409-417.

BIOPHYSICAL STUDIES OF WILD CUCUMBER MOSAIC VIRUS[1]

J. B. Sinclair,[2] P. H. Geil,[3] and Paul Kaesberg

SUMMARY

When partially purified preparations of wild cucumber mosaic virus were examined in an ultracentrifuge, 2 schlieren peaks were observed, suggesting that 2 components were present. Such virus suspensions layered on sucrose density-gradient columns produced 2 opaque layers after high-speed centrifugation. The upper layer was the ultracentrifugal top component, the lower layer was bottom component. Infectivity studies showed that without exception the bottom component was infectious whereas the top component was not. Ultraviolet absorption tests gave absorption curves typical of nucleic acid for both bottom component and whole virus suspensions, whereas top component suspensions appeared to contain little or no nucleic acid. Electrophoresis studies revealed the isolectric point to be pH 6.6 in phosphate buffer. Sedimentation constants were determined to be $S_{20,w}$ (top) = 59.2 S and $S_{20,w}$ (bottom) = 115.5 S. X-ray scattering experiments showed that the bottom component is relatively uniform in density and approximately spherical with a diameter of 284 A. The top component has a dense shell with inner and outer diameters of 210 and 280 A, respectively, the central portion being partly filled with material. Frozen-dried preparations, when examined in the electron microscope, have a particle size of about 280 A for both top and bottom components. Both components appear to be polyhedronal in shape.

Preliminary studies of the biophysical properties of several spherical plant viruses have been made in this laboratory in an effort to find examples in which the infected plant produces, in addition to the virus, a protein related to it. The first such discovery was made by Markham and Smith (5), who showed that the multiplication of turnip yellow mosaic virus is accompanied by the production of a so-called top component protein. The top component was shown to be structurally similar to the protein portion of the virus, and its study, together with that of the virus, has contributed much to our knowledge of virus structures. Similarly, a study of suspensions of squash mosaic virus revealed the presence of 3 high-molecular-weight components (6). Their structural relationship was not determined, although the top component of this virus was found to be not infectious. Wild cucumber mosaic virus, isolated by J. H. Freitag (2) and described by Lindberg, Hall, and Walker (3), has many properties similar to those of squash mosaic virus but is serologically distinct. The multiplication of this virus is accompanied by the production of a large protein particle. Some of the biophysical properties of these 2 components are described below.

PURIFICATION AND SEPARATION OF TOP AND BOTTOM COMPONENTS.—The virus was concentrated and partially purified by acid fractionation followed by alternating high- and low-speed centrifugation in a Model L Spinco ultracentrifuge (3). The final pellets were suspended in 0.1 M sodium phosphate buffer (pH 6.2) and examined in a Svedberg oil turbine ultracentrifuge. Two schlieren peaks were obtained. Identical results were obtained when the virus preparation, in a 0.1 M sodium phosphate buffer at pH 7.0, was examined in a Spinco Model E analytical centrifuge (Fig. 1). These results suggested that the preparation consisted of 2 components, which were designated as top and bottom.

The use of a separation cell in the Spinco analytical centrifuge made it possible to obtain small quantities of top component free from bottom component, but the latter could not be obtained entirely free from the

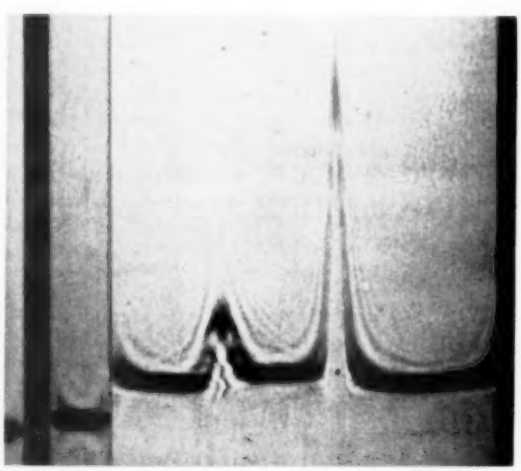

FIG. 1. Sedimentation pattern of wild cucumber mosaic virus. Schlieren peaks in 0.1 M phosphate buffer (pH 7.0) after 24 minutes at 24,630 rpm.

[1] Accepted for publication January 27, 1957.

This investigation was supported in part by the Public Health Service, the Herman B. Frasch Foundation, and the Research Committee of the Graduate School from funds provided by the Wisconsin Alumni Research Foundation. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

We wish to express our appreciation to co-workers for technical advice and suggestions, particularly Dr. S. S. Gothoskar, who collaborated on some of the early phases of this study, and Professor R. M. Bock, both of the Department of Biochemistry, and also Dr. Seymour Van Gundy, Department of Plant Pathology, who prepared some of the virus suspensions. We are also much indebted for their counsel to Professor J. C. Walker, Department of Plant Pathology, and to Professor W. W. Beeman, Department of Physics.

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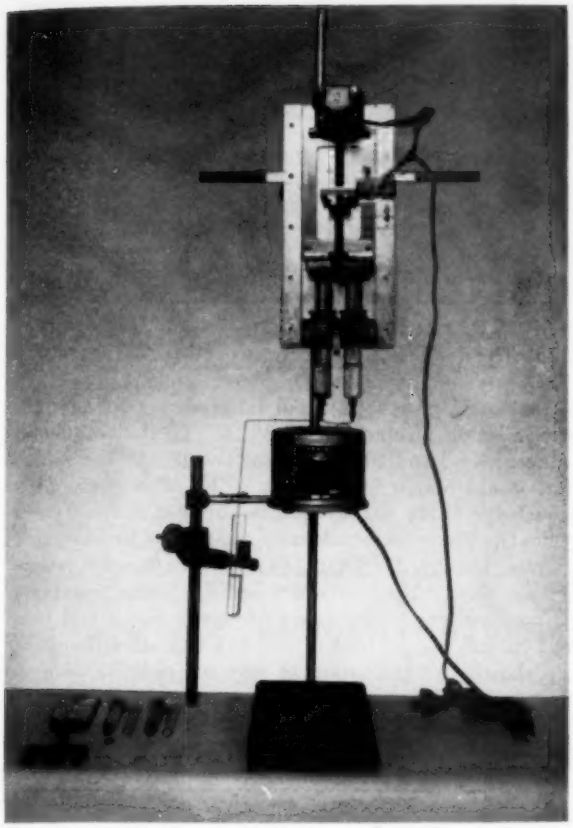

FIG. 2. Apparatus used for preparing sucrose density-gradient columns. A 5-rpm motor forces both syringes down evenly. The more concentrated sucrose solution from the right is mixed with the less concentrated on the left by means of the magnetic stirrer, and the resulting solution is forced into the centrifuge tube.

top component. Infectivity studies made on pumpkin (*Cucurbita pepo* L. var. Small Sugar) showed that a suspension containing mostly bottom component (with a small admixture of top component) remained infectious, whereas one containing only the top component did not.

The limitations imposed by the separation cell were overcome with sucrose density gradients similar to those described by Brakke (1). Density-gradient columns of sucrose dissolved in 0.1 M sodium phosphate buffer (pH 5.0 or 7.0), were made with an apparatus (Fig. 2) designed by Professor R. M. Bock. Two 20-ml syringes resting on sponge rubber rings were held in place with a Lucite block. A threaded rod attached to a 5-rpm motor allowed for slow turning and equal pressure on the syringe plungers. A 40 per cent sucrose solution was forced from the syringe on the right through a modified hypodermic needle into the syringe on the left, which contained 5 per cent sucrose, and there was agitated by means of a small magnetically operated stirrer. The resulting sucrose mixture was forced out and down to the bottom of the centrifuge tube through a hypodermic needle. At the beginning, only 5 per cent sucrose was deposited there. The more concentrated sucrose solution lifted the less concentrated solution and resulted in the formation of a smooth gradient ready for immediate use. Approximately 2 ml of a concentrated virus suspension was layered on top of each column by means of a hypodermic needle and syringe. These tubes were then centrifuged immediately at 25,000 rpm for approximately 2 hours in a Spinco Model L ultracentrifuge equipped with a swinging bucket rotor, No. SW25.1. When the tubes were removed and placed directly under an incandescent bulb, 2 opalescent layers were observed in the gradient column, the lower being less than halfway down, the other about 1/3 the way down. A clear layer consisting of buffer remained on top of the gradient column. An increase in the length of centrifugation did not materially increase the separation of the 2 components. The lower layer proved to be bottom component, the upper layer to be top component. These layers were removed separately with a syringe attached to a J-shaped hypodermic needle brought to rest under each layer. In no case did concentrated healthy-plant sap, taken through the same procedure, show any opalescent layering.

After each separation, 9 seedling plants of pumpkin were inoculated with each of the 2 components; without exception, the top component suspension was not infectious, whereas all plants inoculated with the bottom component developed symptoms typical of those incited by the whole virus.

ULTRAVIOLET ABSORPTION.—Both bottom component and whole-virus suspensions, when examined in a Cary Model 11 recording spectrophotometer, showed absorption at 260 mμ typical of nucleic acid. Absorption curves of top component suspensions had a broad plateau in the neighborhood of 270 mμ, suggesting that they contained little or no nucleic acid (Fig. 3).

ELECTROPHORESIS.—Samples of the concentrated

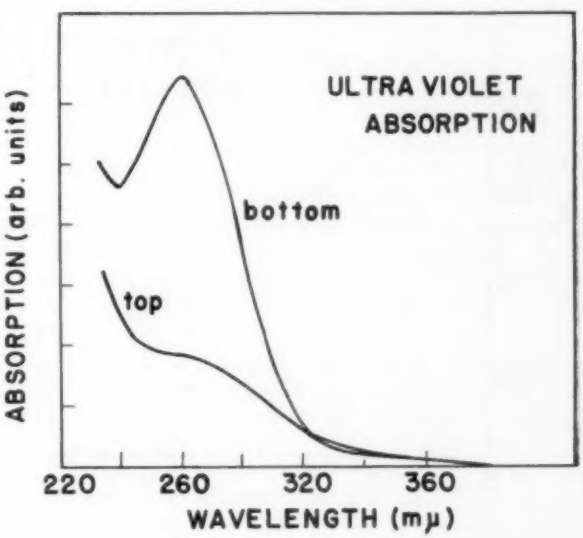

FIG. 3. Ultraviolet absorption of top component (lower curve) and bottom component (upper curve) suspensions of wild cucumber mosaic virus.

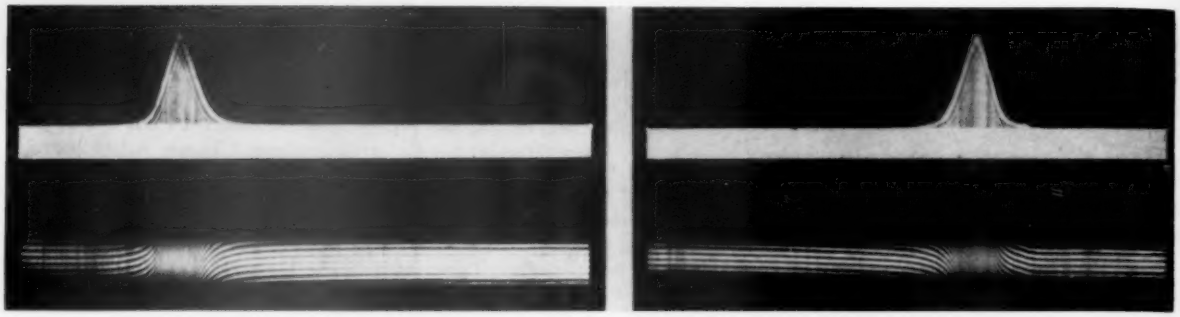

FIG. 4. Electrophoretic pattern of wild cucumber mosaic virus. Schlieren peaks and Rayleigh interference fringes in 0.1 M phosphate buffer (pH 7.0) after 392 minutes at 0.275 volts/cm. The descending boundary is on the left.

whole virus were examined in a microcell in a Spinco Model H electrophoresis apparatus at pH 5.0, 6.0, and 7.0 in 0.1 M sodium phosphate buffer and at pH 8.0 in tris(hydroxymethyl)aminomethane buffer. The virus did not remain infectious at pH 8. At no time, even during runs lasting 7 hours or more, was more than 1 virus peak resolved (Fig. 4); however, thermal convection spikes were evident in several of the runs. At pH 8.0, the single peak was wide and very asymmetric. The values of μ, the average electrophoretic mobilities of the ascending and descending boundaries, were calculated from the conductance of the buffer (Fig. 5). From these values, the isoelectric point in the phosphate buffer system used was determined to be pH 6.6.

SEDIMENTATION.—Sedimentation coefficients for the whole-virus suspensions were measured in a Spinco Model E ultracentrifuge at 21.0°C in 0.1 M sodium phosphate buffer (pH 7.0) at 24,630 rpm. These data were plotted to show the relation of $ln\ x$ to t where x is the distance of the boundary from center of rotation at time t (Fig. 6). Extrapolations were made to infinite dilution in buffer (Fig. 7). The extrapolated values, 54.4 S and 107.1 S, were corrected to the values that would obtain in water at 20°C. This required a knowledge of the partial specific volumes of the 2 components. These were taken arbitrarily to be the same as reported by Markham (4) for the turnip yellow mosaic virus. The corrected sedimentation constants (S = Svedberg units) are as follows: $S_{20,w}$ (top) = 59.2 S, and $S_{20,w}$ (bottom) = 115.5 S. Healthy-plant sap showed no indication of any sedimenting component.

Samples from the 3 layers seen in the density-gradient column also were examined. The sample from the

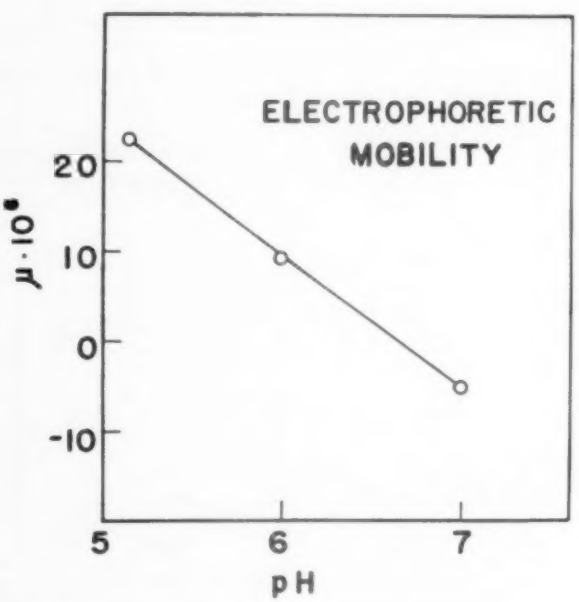

FIG. 5. Electrophoretic mobility of wild cucumber mosaic virus as a function at pH. Circles are averages of ascending and descending mobilities.

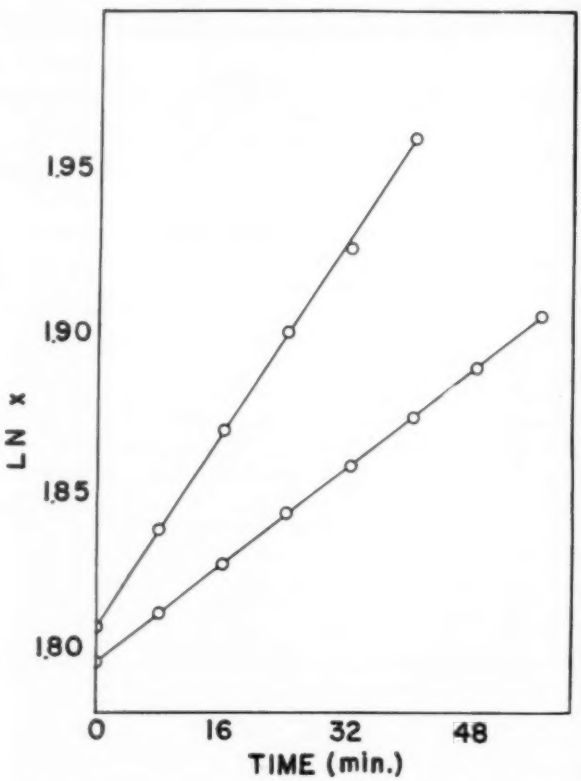

FIG. 6. Displacement of boundaries of top and bottom components of wild cucumber mosaic virus as a function of time.

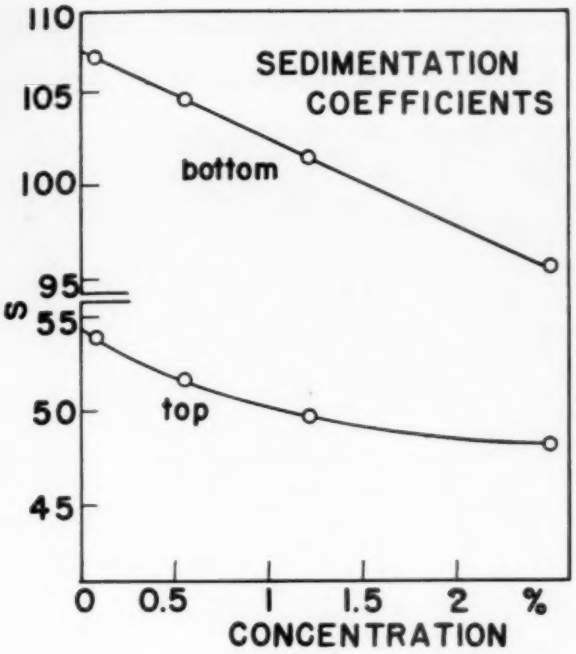

FIG. 7. Extrapolation of sedimentation coefficients as a function of concentration. The concentration given is that of the bottom component as measured by ultraviolet absorption. Concentration of top component is proportional to that of bottom. Solid line extrapolations give $S_{top} = 54.4$; $S_{bottom} = 107.5$.

topmost clear layer showed no sedimenting component at speeds up to 59,780 rpm. This layer was considered to be the buffer in which the virus was originally suspended. The sample from the middle layer consisted of pure top component, whereas that from the bottom layer showed bottom component together with some remaining top component that was almost completely removed by a second separation in the density-gradient cell.

When the virus dissolved in pH 8.0 buffer described above was centrifuged, only 1 peak was observed; the rate of sedimentation corresponded to that of the top component.

DIFFUSION.—An estimate of the diffusion constant D for a suspension containing both top and bottom components was made by permitting an extremely sharp ascending boundary, formed near the end of an electrophoresis run, to diffuse for more than 10,000 minutes. Values for D were determined from photographs of the Rayleigh interference fringe taken at 4900, 7060, and 10,025 minutes by the method described by Svensson (8). The effect of the initial buffer gradient should be negligible after the above lengths of time. Of a total of 13.4 fringes on the photographs, 9 or 10 of those in the center of the pattern fell on a straight line on a plot of coordinate of the fringe versus the integral of the error function. The value of D was calculated from the difference in the squares of the slopes of the lines from 2 photographs in order to eliminate the difficulty in determining the starting time. The average of the 3 possible values determined in this manner was 5.8×10^{-8} cm^2/second at a temperature of 2°C. This gives $D_{20,w} = 1.1 \times 10^{-7}$ cm^2/second. This result is the right order of magnitude for a particle with a diameter of 300–400 A; however, its value is not accurate enough to warrant calculation of particle diameters or molecular weights.

X-RAY SCATTERING.—The angular distribution of X rays scattered from solutions of top and bottom components was measured in order to obtain information about their size and shape (7). By means of a Fourier inversion, the radial electron density of the 2 components was obtained (spherical symmetry was assumed). Plots of the radial electron density indicated that the top component has a dense protein shell with an outer diameter of 280 A and an inner diameter of 210 A. The center is filled with a material less dense than the shell. The bottom component is approximately 284 A in diameter and is nearly uniform in density. If indeed the protein and the virus are structurally related (as are turnip yellow mosaic virus and protein), it would seem necessary to conclude that the nucleic acid is localized within a diameter of 210 A, since in this volume there is considerably less material in the protein than in the virus. A more extensive description of these data is to be published.

ELECTRON MICROSCOPY.—Air-dried and frozen-dried preparations of the top and bottom components were examined in the electron microscope. Samples for air-drying were sprayed with a high-velocity gun onto carbon films mounted on standard specimen screens. Frozen-dried samples were prepared by essentially the method described by Williams (9). Virus suspensions were sprayed onto carbon-filmed screens in contact with a cold brass block (−70°C). Sublimation was carried out at about −40°C. All samples were shadowed with uranium and examined in an RCA electron microscope, Model EMU2.

Air-dried samples of bottom component invariably showed uniform, circularly contoured particles about 370 A in diameter (Fig. 8, A). The shadows indicated a thickness of less than 200 A. Frozen-dried particles were polygonal in contour, the most frequently appearing number of sides being 6 (Fig. 8, B). The particles were about 280 A in diameter and their shadows indicated they were not flattened. The shadows usually exhibited 4 or 5 straight sides, suggesting that the particles have the shapes of polyhedrons with at least 12 faces.

Electron micrographs of air-dried top component usually showed what appeared to be amorphous masses of material together with greatly flattened individual particles with diameters in the neighborhood of 400–450 A and a thickness less than 150 A (Fig. 8, C). Pictures of frozen-dried preparations showed the usual 3-dimensional masses obtained by freeze-drying and also showed polygonally contoured particles of diameter about 280 A (Fig. 8, D). Their shadows often had several straight sides, and their length indicated that

the particles were not flattened. The particles are very likely polyphedronal in shape, but neither their contours nor their shadows were sufficiently consistent in appearance to allow a more definitive statement about their morphology.

It is clear that the air-dried particles of both top

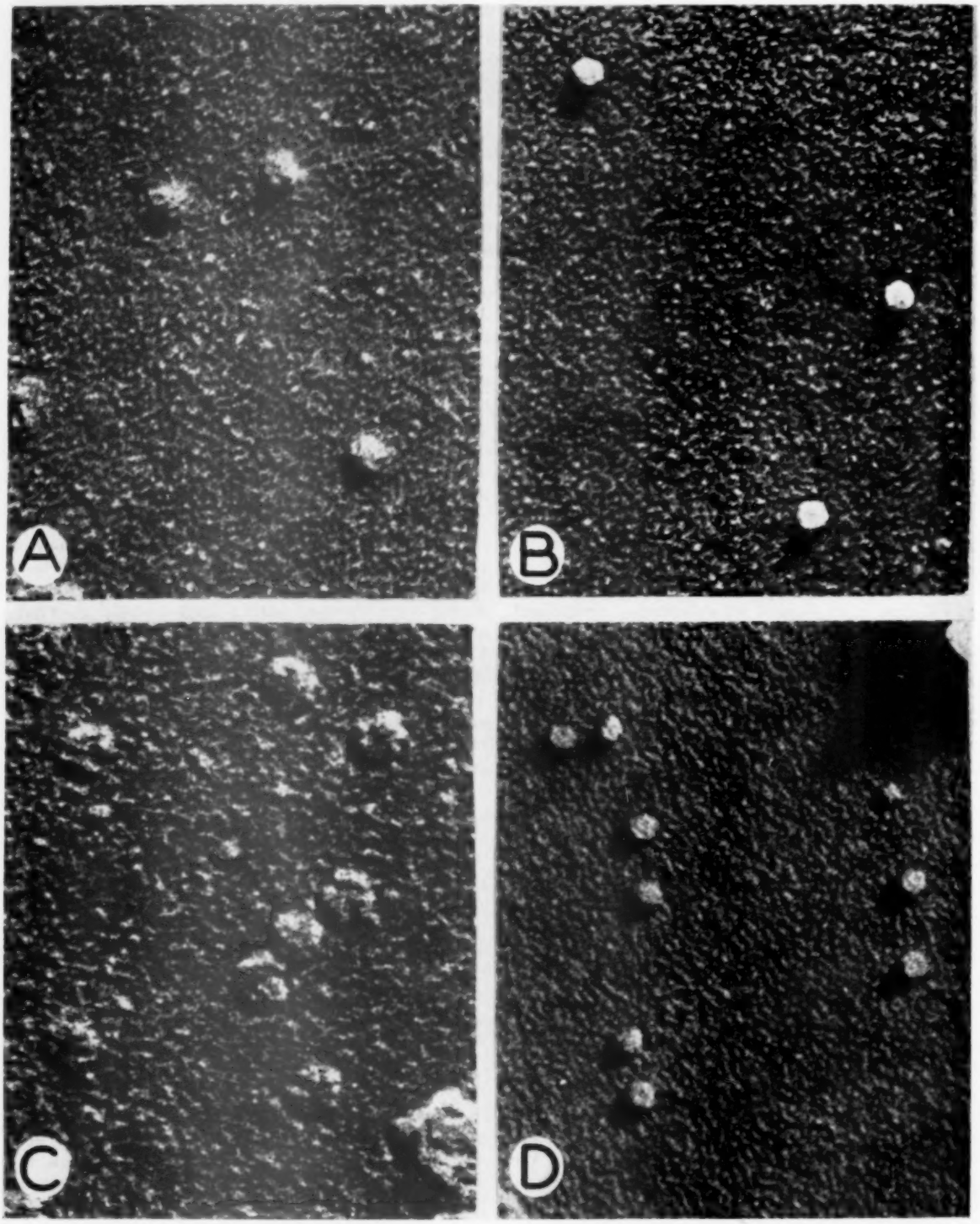

FIG. 8. Electron micrographs of the components of wild cucumber mosaic virus suspensions (about × 180,000). A) Air-dried bottom component. B) Frozen-dried bottom component. C) Air-dried top component. D) Frozen-dried top component.

and bottom component have been severely flattened by surface tension effects.

DISCUSSION.—The studies described above show quite clearly that the bottom component of wild cucumber mosaic virus suspensions has physical properties similar to those of several well-known so-called spherical viruses. Its ultraviolet absorption corresponds to that of nucleoprotein. The component gives an X-ray scattering curve characteristic of a nearly spherical particle with diameter of about 284 A. Electron micrographs yield a similar dimension and suggest a polyhedronal shape. Infectivity is associated with this component, and it can be assumed for the present at least that it is the wild cucumber mosaic virus.

The role played by the top component is less obvious. Its physical properties make it attractive to conjecture that it bears the same structural relationship to the bottom component as does the top component of turnip yellow mosaic virus to its bottom component. That is, the top component is a nucleic-acid-free protein similar in structure to the protein portion of its bottom component. Its ultraviolet absorption corresponds to a protein that is free of nucleic acid. Data on X-ray scattering indicate that it is a semihollow sphere with inner and outer diameter of 210 and 280 A, respectively. Electron microscopy yields a similar external diameter of 280 A with a hint of polyhedronal structure; however, there is no electron microscopic evidence for a hollow structure. The electrophoretic experiments are consistent with the assumption of similar superficial structure for the top and bottom component particles.

The evidence for the relationship between top and bottom components is by no means conclusive. It is sufficiently suggestive, however, to warrant more extensive biophysical experiments as well as serological and chemical analyses when more material becomes available.

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LITERATURE CITED

1. BRAKKE, M. K. 1953. Zonal separations by density-gradient centrifugation. Arch. Biochem. Biophys. **45**: 275-290.
2. FREITAG, J. H. 1952. Seven virus diseases of cucurbits in California. (Abs.) Phytopathology **42**: 8.
3. LINDBERG, G. D., D. H. HALL, AND J. C. WALKER. 1956. A study of melon and squash mosaic viruses. Phytopathology **46**: 489-495.
4. MARKHAM, R. 1951. Physiochemical studies of the turnip yellow mosaic virus. Discussions Faraday Soc., No. 11, p. 221-227.
5. MARKHAM, R., AND K. M. SMITH. 1949. Studies on the virus of turnip yellow mosaic. Parasitology **39**: 330-342.
6. RICE, R. V., G. D. LINDBERG, P. KAESBERG, J. C. WALKER, AND M. A. STAHMANN. 1955. The three components of squash mosaic virus. Phytopathology **45**: 145-148.
7. SCHMIDT, P., P. KAESBERG, AND W. W. BEEMAN. 1954. X-Ray scattering from turnip yellow mosaic virus. Biochim. et Biophys. Acta **14**: 1-11.
8. SVENSSON, H. 1951. On the use of the Rayleigh-Philpot-Cook interference fringes for the measurement of diffusion coefficients. Acta Chem. Scand. **5**: 72-74.
9. WILLIAMS, R. C. 1953. A method of freeze-drying for electron microscopy. Exp. Cell Research **4**: 188-201.

A PHYTOPATHOLOGICAL NOTE

Organic Acids and the Control of Two Seedling Blights by the Water-soak Method.[1] P. JACQUET, CURT LEBEN[2], AND D. C. ARNY. In continuation of work reported by Leben, Scott, and Arny on the nature of the mechanism of the water-soak method[3], it seemed of interest to test further the organic acids found in the steep-water by attempting to control seed-borne diseases through treatment of seed with these acids in pure form. Two seedling blights incited by *Helminthosporium victoriae* Meehan & Murphy and *H. sativum* Pam., King, & Bakke, in oats (Vicland, C.I. 3611) and barley (Wisconsin Barbless, C.I. 5105), respectively, were employed. Both diseases have been reported to be controlled by the water-soak method.[4]

Seed was naturally infected with the organisms. One part of seed was soaked in 5 parts of the test solution (weight/volume). Variables included in the treatments were length of soaking time, temperature during the soaking period, and pH and concentration of the solution. Since the metabolic activity of microorganisms during the soaking period had been implicated in the production of the acids,[3] tests with these materials were conducted under conditions unfavorable for microbial activity, i.e. a short soaking period or a low temperature. After treatment, seed was dried and then sown in flats in the greenhouse. There were 3 replicates of 50 seeds for each treatment in each

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation.

[2] Present address, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Indiana.

[3] Leben, C., R. W. Scott, and D. C. Arny. 1956. On the nature of the mechanism of the water-soak method for controlling diseases incited by certain seed-borne pathogens. Phytopathology 46: 273-277.

[4] Arny, D. C., and C. Leben. 1956. Control of several small grain diseases by the water-soak seed treatment. Phytopathology 46: 344-345.

TABLE 1.—*Germination of seeds and disease incidence in seedlings following the soaking of oat seed, infected with Helminthosporium victoriae, and of barley seed, infected with H. sativum, in a synthetic mixture of organic acids*[a] *in water*

Treatment	Time in hours	°C	pH of "synthetic"	Concentration of "synthetic"[b]	Oat seed infected with *H. victoriae*		Barley seed infected with *H. sativum*	
					Percentage germination	Percentage diseased seedlings	Percentage germination	Percentage diseased seedlings
None					92	24	94	92
Ceresan M					94	1	99	2
Water	56	24			80	8	85	45
Synthetic	56	2	5	20×			84	38
				10×	83	1	94	52
				5×	85	3	83	59
				2.5×	81	8	93	81
	56	2	6	40×	81	2	91	66
				20×	79	2	91	74
				10×	88	6	92	72
	24	2	5	20×	65	1	89	31
				10×	89	4	92	49
			6	20×	85	4	90	74
				10×	90	11	96	79
	4	2	5	40×	25	6	89	35
				20×	87	3	85	50
			6	40×	86	5	87	57
				20×	86	6	89	63
	4	24	5	40×	27	3	82	45
				20×	80	3	89	45
			6	40×	88	7	93	65
				20×	91	9	88	71
	2	24	4	20×			70	50
				10×			90	74
	2	24	5	40×			80	59
				20×			96	81
				10×			97	93
	2	24	6	40×			93	78
				20×			97	81
				10×			96	87
LSD[c] (P = 0.05)					7	5	8	11

[a] Synthetic was composed of 98 per cent formic acid, 0.05 ml/l; glacial acetic acid, 0.12 ml/l; *n*-butyric acid, 0.18 ml/l; succinic acid, 15 mg/l; and absolute ethanol, 1.0 ml/l.

[b] A 1× concentration contained the approximate amounts of organic acids found in filtrates from soaked seeds.

[c] Ceresan M data not included in statistical analysis.

test.

The acid solution "synthetic" contained the approximate amount of organic acids found in filtrates from soaked seed.[3] This mixture was composed of the following: 98 per cent formic acid, 0.05 ml/l; glacial acetic acid, 0.12 ml/l; *n*-butyric acid, 0.18 ml/l; succinic acid, 15 mg/l; and absolute ethanol, 1.0 ml/l. It should be noted that in the "synthetic" used for spore germination studies,[3] no ethanol was added; however, since some ethanol had been detected in the filtrates, it was decided to use it in the "synthetic" for the experiments here described. The pH of the solution was adjusted with NaOH.

The results of these tests are compiled in Table 1. Ceresan M (7.7 per cent *N*-(ethylmercuri)-*p*-toluenesulfonanilide), considered as a control, was effective against both blights when applied at the recommended rate of 0.5 oz. per bushel. The effect of "synthetic" was dependent on 1) concentration, 2) pH, 3) length of the soaking period, and 4) the temperature during the soaking period. For oats, several combinations were effective. A treatment period of 56 hours at 2°C gave control at a 5× or 10× concentration at pH 5, or with 20× or 40× at pH6. With a shorter treatment period, 24 hours, 10× at pH5 or 20× at pH6 were required for control. For a still shorter treatment period, 4 hours, 20× or 40× were necessary at pH5 or 6. For the control of the barley seedling blight, these same general relationships were observed. It is concluded, therefore, that as the pH of "synthetic" is lowered, less of it is needed for disease control or a shorter treatment period will suffice. Concomitant with the lowering of the pH, however, is an increase in phytoxicity to both plant species (Table 1).

The work in this paper is taken as further evidence of the importance of organic acids as factors in the effectiveness of the water-soak treatment for the control of certain diseases incited by seed-borne pathogens.—Departments of Plant Pathology and Agronomy, University of Wisconsin, Madison.